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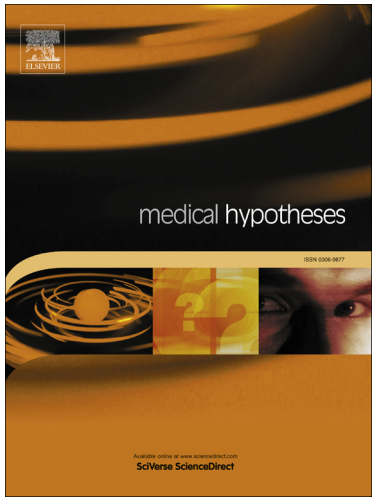

The balance between the pro-inflammatory effect of plasma noradrenaline and the anti-inflammatory effect of neuronal noradrenaline determines the peripheral effect of noradrenaline

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The balance between the pro-inflammatory effect of plasma noradrenaline and the anti-inflammatory effect of neuronal noradrenaline determines the peripheral effect of noradrenaline.

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CONFLICTs OF INTEREST

- none

SUMMARY.

Perfusion experiments on an isolated, canine lateral saphenous vein segment preparation have shown that noradrenaline causes potent, flow dependent effects, at a threshold concentration comparable to that of plasma noradrenaline, when it stimulates the segment by diffusion from its microcirculation (vasa vasorum). The effects caused are opposite to those neuronal noradrenaline causes in vivo and that, in the light of the principle that all information is transmitted in patterns that need contrast to be detected – star patterns need darkness, sound patterns, quietness – has generated the hypothesis that plasma noradrenaline provides the obligatory contrast tissues need to detect and respond to the regulatory information encrypted in the diffusion pattern of neuronal noradrenaline. Based on the implications of that hypothesis, the controlled variable of the peripheral noradrenergic system is believed to be the maintenance of a set point balance between the contrasting effects of plasma and neuronal noradrenaline on a tissue. The hypothalamic sympathetic centres are believed to monitor that balance through the level of afferent sympathetic traffic they receive from a tissue and to correct any deviation it detects in the balance by adjusting the level of efferent sympathetic input it projects to the tissue. The failure of the centres to maintain the correct balance, for reasons intrinsic or extrinsic to themselves, is believed to be responsible for degenerative and genetic disorders. When the failure causes the balance to be polarised in favour of the effect of plasma noradrenaline that is believed to cause inflammatory diseases like dilator cardiac failure, renal hypertension, varicose veins and aneurysms; when it causes it to be polarised in favour of the effect of neuronal noradrenaline that is believed to cause genetic diseases like hypertrophic cardiopathy, pulmonary hypertension and stenoses and when, in pregnancy, a

factor causes the polarity to favour plasma noradrenaline in all the maternal tissues except the uterus and conceptus, where it favours neuronal noradrenaline, that is believed to cause preeclampsia.

INTRODUCTION

There is virtually universal agreement that plasma noradrenaline (PNA) at its basal concentration of 250-300 pg/ml [1] has no functional effect and, consequently, that any effect associated with noradrenaline (NA) is attributable solely to the drug's neurotransmitter function [2]. Consistent with that belief, the threshold concentration at which intraluminal NA constricts the canine lateral saphenous vein (CLSV) segment is 0.2 µM (34.8 ng/ml) NA [3] and the minimum concentration at which NA causes a detectable effect when infused into healthy subjects is 1,800 pg/ml [2]. However, the belief PNA has no effect at its basal concentration raises serious questions. Why, for instance, has the evolutionary process conserved neuronal noradrenaline (NNA) overspill and PNA uptake if PNA has no function? why does adrenaline(ADR) cause dramatic effects at a concentration of around 170 pg/ml and PNA cause none at 250 pg/ml plus? why has PNA been shown to be capable of causing *supra* threshold dilator effects on tonically constricted CLSV segments at a concentration that may have been as low as 590 pg/ml?[4]. And, finally, and most cogently, why do the valves of veins responsive to NA, and only those valves [5], have a structure called an agger that forms the core element of a mechanism that enables reflux PNA lower a vein's elevated tone by stimulating it through diffusion from its microcirculation [6]. Aggers have been known to exist for more than two centuries [7] but because it was never possible to get an agreement on their function their existence has now been virtually forgotten, as evidenced by the fact that most medical dictionaries contain no reference to them and any current textbook I am aware of, including Gray's Anatomy, makes no mention of them. In my opinion the existence of valve aggers is as good an

indication that PNA has a significant functional role as the existence of the valves themselves is an indication that the movement of blood is circular and not tidal.

The aim of this three part paper is two-fold,(1) to demonstrate that PNA has a functional role as important as that of NNA and (2) to explain how that role operates in various clinical disorders. The first part of the paper describes the effects exogenous NA and overspill NA have on isolated segments of the canine lateral saphenous vein (CLSV) and that contrast with the effects NNA causes in vivo. However, because those effects cannot be duplicated experimentally in vivo, because of a masking effect of efferent sympathetic activity, they have beendismissed, up to now, as experimental artifacts. The second part of the paper uses the findings described in part one as the basis for two constructs, (1) a hypothesis that PNA stimulation provides the contrast or, in a biological context, the lateral inhibitory effect adrenergically innervated tissues need to detect and respond to the regulatory information encrypted in the spatio-temporal diffusion pattern of NNA and, (2) a paradigm of the peripheral noradrenergic system(NAS) that states the controlled variable of the system is a set point ratio or balance between the contrasting effects of PNA and NNA on a tissue. The system is believed to be controlled by the hypothalamic sympathetic centres that monitor the PNA/NNA balance of a tissue through the level of afferent stimulation it receives from the tissue and corrects any deviation it detects in the balance by adjusting, up or down, the level of efferent sympathetic activity it projects to the tissue. The third part of the paper analyses the aetiology of several pathological conditions in the light of the new hypothesis and related NAS paradigm. That analysis has concluded that any factor that disturbs the balance between the effects of PNA and NNA on a tissue causes remodeling changes in the tissue. When a factor shifts the balance in favour of the pro inflammatory effect of PNA,

that is believed to cause conditions like dilator cardiac failure, varicose veins, hypertension and aneurysms; when it shifts it in favour of the growth promoting, anti-inflammatory effect of NNA that is believed to cause conditions like hypertrophic cardiopathy and stenoses, and when, in pregnancy, it shifts it in favour of PNA in the mother overall, but not in her uterus or conceptus, where it shifts it in favour of NNA, then that is believed to cause preeclampsia.

The bulk of the experiments on whose findings the new hypothesis and paradigm are based, were conducted prior to the discovery of nitric oxide (NO) or to its role being fully appreciated. In the circumstance, no formal attempt was made at the time to determine if NO might have been responsible for the flow dependent vasodilator effects associated with PNA stimulation. However, the evidence (v.i.) is strongly against that having been the case. In addition, little effort was made to classify the various effects of NA on the CLSV segment pharmacologically because of the early evidence that the drug sometimes caused qualitatively different effects in different parts of the segment at the same time. In the circumstance the use of blocker drugs was considered to be potentially confusing and not worthwhile, particularly as I employed a technique (v.i.) that made it possible to visibly observe the precise nature, extent and location of the effects NA caused.

PART 1

The first evidence I had that exogenous and overspill endogenous NA could cause dilator effects emerged during a routine pharmacological investigation of the responses of a perfused, doubly cannulated, in vitro preparation of segments of the CLSV to NA (Levophed) and electrical stimulation [8],[9]. Early in the course of the investigation an occasional segment displayed bimodal responses to stimulation [10] and that, in the context of the constant flow perfusion technique being employed, implied exogenous NA and overspill NA were causing dilator as well as constrictor effects. Initially, the bimodal responses were dismissed as experimental artifacts. However, because of a prior clinical interest I had in varicose veins I was reluctant to accept that verdict off hand: might it be possible a dilator effect of PNA was responsible for the varicosities of varicose veins? I accepted that suggestion would have to be treated with caution because if NA was causing a dilator effect then, it seemed, it had to be doing so by stimulating the segment by diffusion from its microcirculation. However, as far as was known at the time, no one had ever been able to perfuse the microcirculation of a healthy vein by reflux and consistent with that fact, a widely cited article [11] claimed that the microcirculation of a vein drains into the lumens of neighbouring veins and not into the lumen of its host, implying no channel existed for anything perfusing the lumen of a healthy vein segment to perfuse the segment's microcirculation.

However, shortly after becoming aware of the bimodal responses I made a series of speculative 3ml injections of isoprenaline (ISO), a proven intraluminal dilator of the CLSV segment [9], into the distal end of a major tributary of the segment and found they caused dose related, localised constrictor effects [12]. The fact the responses were located

upstream from where the injected tributary emptied into the perfused segment (fig 1) and upstream also of a competent valve in the lumen of the segment, meant it was impossible for ISO to have constricted the segment by an intraluminal effect at the constricted site. By exclusion, the only alternative seemed it must have done so by a reflux microcirculatory effect.

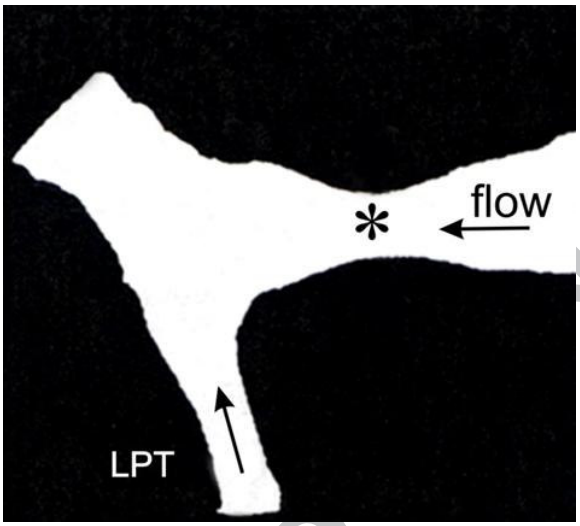

Fig 1.

Localised constriction (*) of a perfused CLSV segment caused by isoprenaline injected into the Lateral Plantar tributary (LPT).

That that was indeed the case seemed to be confirmed when a solution of ISO and ink were injected into the tributary and the ink stained the microcirculation of the perfused segment only where ISO constricted it [13]. That established that, contrary to prevailing belief, a channel existed between the lumen of the tributary and microcirculation of the CLSV segment and that exogenous and overspill NA had probably used it to cause the dilator effect of their associated bimodal responses. And since ISO had caused its constrictor effect at a threshold concentration of ~ 1ng/ml,[12][13], which is 30-40 times lower than the ~

0.2µM (34.8 ng/ml) threshold at which NA caused its intraluminal constrictor effect [14], it was conceivable that PNA, at its basal concentration of around 250-300 pg/ml, could dilate the segment by stimulating it by diffusion from its own microcirculation. That possibility was strengthened by the evidence that the concentration of overspill NA associated with standard levels of electrical stimulation of the canine lateral saphenous vein in vivo was comparable to the basal concentration of PNA in the human saphenous vein [15]. If microcirculatory PNA stimulation was responsible for causing the varicosities of varicose vein then that implied it must have a pro inflammatory effect [16].

Material and methods.

Isolated CLSV segments were obtained from large dogs, preferably greyhounds, put down by electrocution [9]. Initially, only in vitro segments were employed but after 2-3 years in situ segments were far more commonly employed. In situ preparations of combined segments of the canine cranial tibial artery and vein were obtained in the same way as the in situ CLSV segments.

After being doubly cannulated, segments were perfused with oxygenated Tyrode solution at 34°C, at constant flow rates of between 35 and 40 ml/min, with drugs being added to the perfusion reservoir or injected into the perfusion circuit through a side tap located close to the upstream cannula. The responses of segments were monitored in two ways: by recording the pressure changes in the perfusion circuit, when a rise in pressure indicated a segment was constricting overall and a fall indicated it was dilating overall, and by fixing segments with a rapidly acting primary fixative injected into the perfusion circuit as their responses reached their peak and subsequently observing the structural changes associated

with the responses. The fixative consisted of a pH 6.6-6.8 solution of 2% glutaraldehyde and 2.5% paraformaldehyde in phosphate buffer.

When needed, the distributive effect of turbulence was used to increase flow, and the effect of drugs, in randomly located or preselected microcirculatory modules. Several means were used to induce the turbulence but the most effective and frequently used was floating of a length of knotted domestic sewing thread in the perfusate of a segment [17]. When drugs were injected intralumenally, rather than added to the perfusate, turbulence was then induced by making the injections in an abrupt, pulsatile manner.

Over one hundred valves in the lumen and tributaries of CLSV segments were examined histologically to elucidate the structure of the valve agger and the arrangement of the smooth muscle bundles associated with it. All tissues investigated were fixed by the rapidly acting fixative referred to and were serially sectioned, effectively, in random planes at a thickness of 7µm, usually. The bases of several cardinal (terminal) valves in the major tributaries were also examined by scanning electron microscopy after their cusps had been resected.

Results

The most significant of the early experimental finding was the evidence that the microcirculation of a CLSV segment could be perfused by reflux at a pressure as low as 33mm Hg when the segment was stimulated by NA and its flow turbulent [18]. That meant it was, in theory at least, possible that blood could perfuse a vein's microcirculation by reflux from its lumen at physiological pressures. Another significant early finding was the evidence that an estimated minimum 80% of the blood draining from the microcirculation

of the CLSV segment first drained into the bases of the cardinal (terminal) valves of the segment's tributaries before draining into the segment's lumen. That information made it possible to block a minimum 80% of any reflux from the lumen of the CLSV segment perfusing the segment's microcirculation by simply putting ties on all the tributaries of a segment flush with their junctions with the segment. When that was done it had two immediate and highly significant effects: it abolished the occasional bimodal responses and it strengthened the segments' constrictor responses to exogenous NA and, more significantly, electrical stimulation by around 50%.

Another significant early finding was the evidence that the microcirculation of the CLSV segment was composed of modules, each servicing the needs of a 3-5 diameter long section of the segment. That made each section a functionally independent unit in the sense its response to microcirculatory stimulation is independent of, and potentially different from that of every other section [10].

The final significant finding provided the first clear statistical evidence of the dilator potency of reflux microcirculatory NA stimulation. That was provided by a series of comparisons between the responses of two cohorts of in vitro CLSV segments, one 57 in number, the other 27, to intraluminal NA and electrical stimulation [12]. The 57 segment cohort had no flush ties on their tributaries so their microcirculations received normal levels of reflux while the tributaries of the 27 segment cohort had flush ties and their microcirculations received a maximum 20% of normal reflux. Because of receiving so little reflux, with its dilator effect, the constrictor responses of the 27 segment cohort to NA and electrical stimulation were significantly greater than the responses of the 57 segment cohort. As evidence of that, a range of constrictor responses the 57 segment cohort with no ties achieved with

concentrations of exogenous NA ranging 0.1 to 0.3 µg/ml was achieved by the 27 segment cohort, with ties, with concentrations ranging 0.05-0.1 µg /ml, and a benchmark electrical response the 57 segment cohort needed about 500 impulses to achieve was achieved by the 27 segment cohort with between 250 -300 impulses. In addition, when all 1081 responses of the two cohorts of segments to electrical stimulation were analysed it was found only 13 of the 57 segment cohort (23%) had achieved a minimum of one 80 mmHg rise in perfusion pressure, while 18 of the 27 segment cohort (66%) had achieved that mark.

Because the differences between the two cohorts' responses might be related in some way to the use of in vitro segments an experiment was conducted where eleven matched pairs of in situ segments were used to construct eleven dose response curves to NA, using five concentrations ranging from a threshold of 0.2 µM to 6.0 µM, and where the tributaries of one of each pair had flush ties blocking reflux [14]. While there was no significant difference between the paired segments at 0.2 µM, there were significant differences at every other concentration, with the segments with flush ties and blocked reflux, displaying mean constrictor responses 49 to 54% higher than the responses of the segments without ties**.** In brief**,** regardless of whether in vitro or in situ CLSV segments were involved, an estimated minimum 80% reduction in reflux microcirculatory NA perfusion was associated with increased constrictor responses of around 50%.

Aggers and reflux.

The evidence that the junctional regions of tributaries were involved in some way with a segment's constrictor response to ISO and reflux naturally led to their investigation. An initial inspection revealed that virtually every tributary, down to a diameter of around 150-200 microns, possessed a cardinal (terminal) valve located 2-3 tributary diameters from the

tributary junction. However, consistent with belief then, and currently, that valves are cul-de-sacs, inspection revealed no evidence of any venule opening into the base of the valve or, for that matter, into the junctional region. However, on one occasion,when preparing a segment for cannulation, I accidentally made an incision into the sinus of a cardinal valve and saw blood flowing freely, against gravity, from the base of the valve into the lumen of the CLSV for up to twenty minutes. Having seen that, I then used electron microscopy to detect the opening from which the bleeding had taken place [4]. Initially, microscopy was carried out on the valves of segments that were unstimulated by NA at the time of fixation and it found no evidence of any vessel opening into the base of a valve. Instead, all it found was a series of deep folds at the bases of the valves (fig 2A). However, later, when segments that had been stimulated by NA at the time of fixation were examined, microscopy revealed an entirely different picture: all the folds were gone, the bases of the valve were smooth and at the base of each valve was a prominent opening, about 100 microns in diameter (fig 2B). It was apparent on review that the opening detected in the second set of micrographs had been present in the first also but was compressed and hidden in the valve's deep folds (2A).

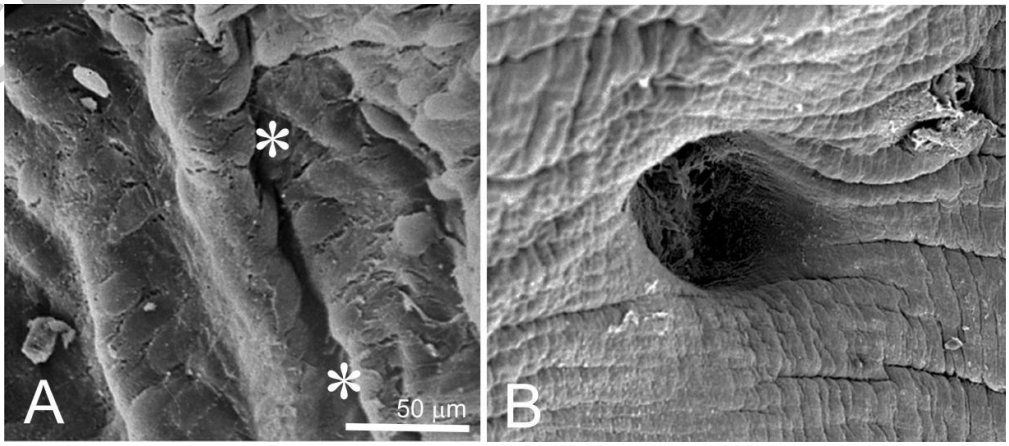

Fig 2. SEM micrographs of the bases of cardinal valves showing (a) the opening of a venule (*-*) compressed by the recoil of an agger and (b) the decompressed opening of a venule when a CLSV segment was stimulated by 10µM NA and the valve agger was consequently stretched.

Further investigation revealed the folds were caused by the recoil of the valve's agger, a dense fibroelastic linear structure, located along the line of attachment of the valve cusp and currently believed to be a mere hinge attaching the valve cusp to the vein wall. In fact the agger is a functional valve, whose compression effect paradoxically assists microcirculatory drainage [19], that prevents a venous microcirculatory module being perfused by reflux when a vein's tone is normal. The effectiveness of the agger in doing that was demonstrated by an experiment where Methylene Blue was injected into the sinuses of cardinal valves at pressures peaking around 200 mmHg and a minimum 92.56 (±3.16) % of the dye was later recovered from the run off of the perfusate within 10 seconds of the injection having been completed [20].

Further investigation revealed the two pairs of smooth muscle bundles project from the walls of a vein and/or its tributaries onto each agger. Because the muscles project onto the agger from opposite directions, they stretch it when they contract as the tone of a vein rises. That decompresses the venules that normally drain microcirculatory modules into valve sinuses and make it possible for the modules to be perfused by reflux when the tone of the vein rises and for the PNA in that reflux to then lower the elevated tone to normal [6][13]. To enable reflux take place, as the tone of a vein rises the A/V shunts of the microcirculatory modules open [17] and divert the arterial inflow. Effectively, the agger is part of a PNA-dependent negative feedback designed to stabilise venous tone.

Further evidence of the dilator potency of microcirculatory PNA stimulation was provided by an experiment where, some 20 minutes post-mortem, Tyrode solution was injected into tonically constricted CLSV segments filled with blood and caused prominent varicosities on the segments (fig 3) [4]. When the experiment was duplicated in segments cleared of blood no varicosities resulted. The concentration of PNA in the blood filling the injected segments varied from 0.59-1.32ng/ml [4].

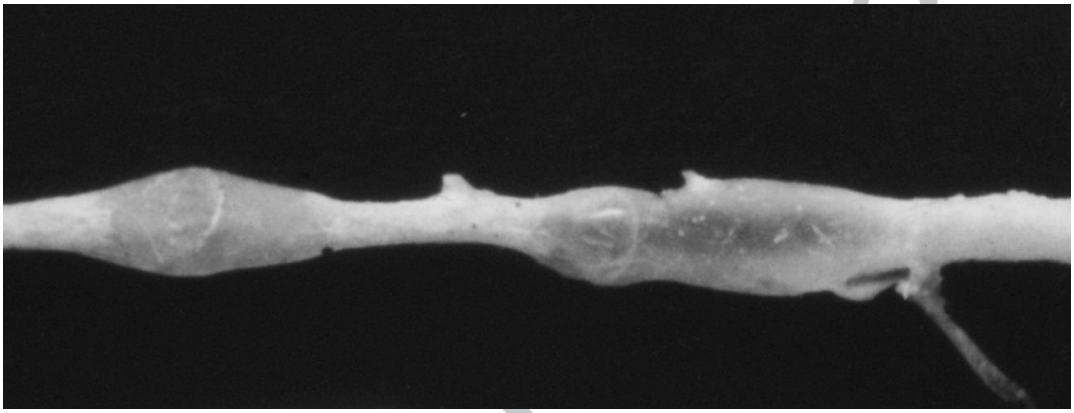

Fig. 3. Varicosities created, some 20 minutes post-mortem, in a tonically constricted CLSV segment by reflux perfusion of the segment's microcirculation with the blood filling the lumen of the segment.

Valve Cannulation.

Having knowledge of how the valve agger operated made it possible to investigate how a 3-5 diameter long section of the CLSV segment, serviced by a single microcirculatory module, responded to drugs stimulating it by diffusion from the module. That investigation was made by injecting drugs into a cannulated sinus of a cardinal valve and noting the response of the section of the segment whose microcirculatory module had been perfused by the

injection. The experiment confirmed the high sensitivity of the section to drugs stimulating it through its microcirculation. A typical experiment involved injecting a 1.0 ml bolus of 1μM ISO into a valve sinus on three occasions and finding it caused a 174 mmHg rise in the overall perfusion pressure of the segment on each occasion [20], compared to the 130 mm Hg rise 6.0 μM intraluminal NA would be expected- by extrapolation from a dose response curve [14] - to cause. Given that aggers block ~ 93% of any drug injected into a valve sinus from perfusing its associated microcirculatory module, the difference in sensitivity to NA stimulating a blood vessel through its lumen and through its microcirculation is, in fact, much greater than the figures quoted indicate. One important implication of that fact is that PNA is a physiological vasodilator and a pharmacological vasoconstrictor .

Another cannulation experiment that again confirmed the dilator potency of microcirculatory PNA involved eliminating the presence of tonically released NA in the microcirculation of a CLSV segment by stimulating the segment with reserpine or guanethidine until it ceased to respond to electrical stimulation and then finding that the segment's constrictor response to a 1ml bolus of 1mM ISO stimulation increased significantly, on one occasion by 91%, from an initial 46 to a subsequent 86 mmHg rise in perfusion pressure[20].

The dilator effects of NO and PNA.

One reason the dilator effect of microcirculatory PNA has not been recognised generally up to now is the high profile currently enjoyed by NO and its involvement in a range of effects, some of them, like PNA, flow dependent and dilator. Because of that profile any inexplicable dilator effect tends to be attributed by default to the action of NO [21] and to the reduced bioavailability of endothelial NO associated with inflammatory disorders. The latter is

currently attributed to a primary dysfunction of the endothelium but is more likely, I believe, to be caused by an inhibitory effect of increased stimulation of the endothelium by PNA in inflammatory conditions . Consistent with that belief the reduced bioavailability of NO in dilator cardiac failure is responsive to the use of the $beta_1$ blocker nebivolol [22].

The coupled segment preparation**.**

When a dog's hind leg is amputated at the knee joint the lumen of the cranial tibial artery is exposed, which makes it possible, by injecting the exposed lumen, to perfuse the microcirculations of both the artery and its companion tibial vein by orthograde flow. When done, it became possible to compare the responses of the cranial tibial artery and vein to orthograde microcirculatory NA stimulation with the responses of the isolated CLSV segment to reflux microcirculatory NA stimulation. That comparison revealed no significant difference between the two in respect of dilating in response to microcirculatory NA stimulation. It also revealed that both arteries and veins in general have the same type of modular construction as the CLSV segment has [23]. Those findings were confirmed when a bolus of 3µM NA was injected in a pulsatile manner into the lumen of a tibial artery and caused a berry aneurysm on the otherwise constricted artery and randomly located varicosities on the tibial vein (fig 4) [23]**.** The fact that the vein was not being perfused at the time the varicosity was created is presumptive evidence that a dilator effect of NO released from the venous endothelium had not been responsible for it.

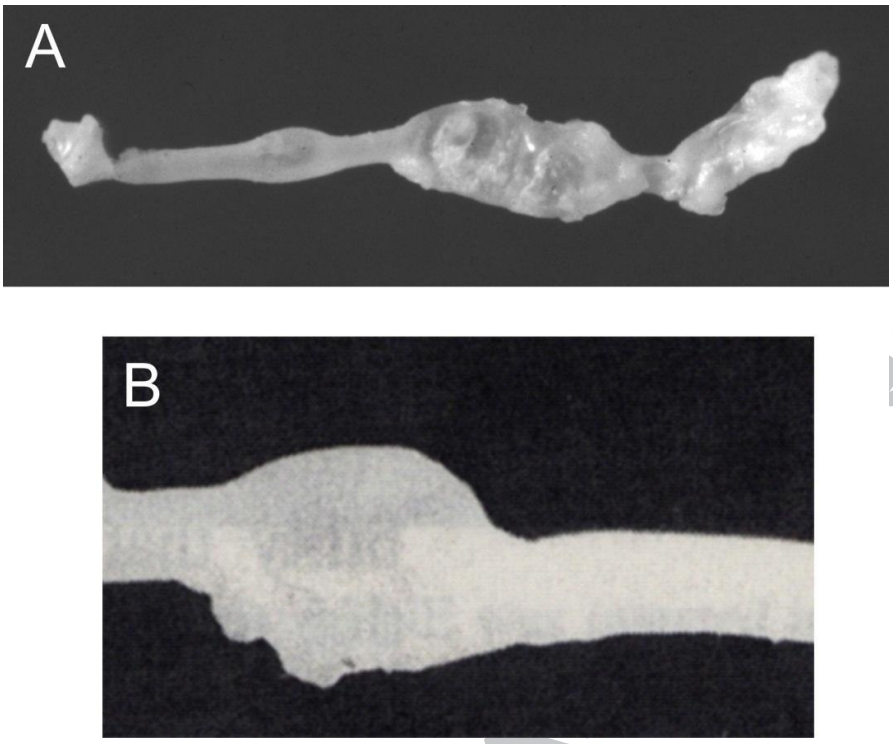

Fig 4. Photographs of (A) varicosities created in a canine cranial tibial vein and (B) a berry aneurysm created in an otherwise constricted canine cranial tibial artery by a pulsatile injection of 3µM NA into the lumen of the artery.

The pro inflammatory effect of microcirculatory PNA.

The belief that increased PNA stimulation has a pro inflammatory effect is based on the evidence that repeated episodes of exogenous NA stimulation of perfused CLSV segments over the course of a day's experimentation caused effects that either mimicked or were predictive of those that occur in varicose veins, a classical inflammatory condition. Among the effects that mimicked those found in varicose veins, and are defining features of that condition, were varicosities, the opening of A/V shunts [17], the formation of blocking intimal cushions in arterioles in the wall of varicosities and the loss of the arterioles distal to the cushions [24][25]. The predictable consequence of that blockage and loss of arterioles

would be tissue hypoxia, another recognised feature of varicose veins [26]. And that hypoxia, in turn, is predictive of yet another recognised feature of varicose veins, viz., a replacement of aerobic [27] by anaerobic respiration [28].

However, the clearest evidence of the pro inflammatory effect of increased microcirculatory PNA simulation was the finding of a fenestrated endothelial cell lining a post capillary venule of the CLSV segment's microcirculation [24]. Even though the fenestration was in the process of closing down at the time of fixation, it was still big enough, had it existed

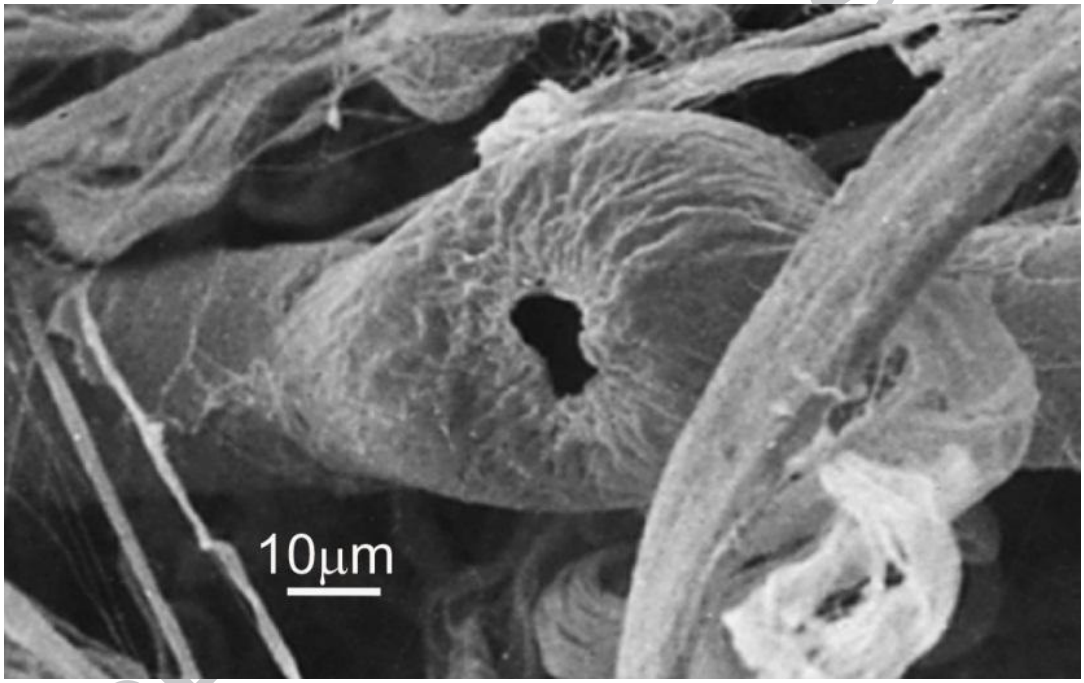

Fig. 5. SEM micrograph of a fenestrated endothelial cell lining a post capillary venule in the microcirculation of a CLSV segment.

in vivo, to allow blood extravascate into the interstitial tissue and cause the haemosiderotic dermatitis common in patients with chronic varicose veins. In the present context, however, the particular significance of the fenestration lies in the fact that it is known to be caused by an increased expression of the pro inflammatory marker TNF-α [29], a ligand with the ability

to cause pro apoptotic inflammatory cascades through activating so called “death receptors” [30].

The neurotoxic effect of increased microcirculatory PNA stimulation.

Assuming increased microcirculatory PNA stimulation is responsible for varicose veins, then its most strategic malign effect is the progressive damage it causes to the adrenergic plexuses of varicosities [31]. That, at some point, would have the effect of making it impossible to maintain the noradrenergic set point ratio of a varicosity because of the inability of its damaged adrenergic nerves to release sufficient NNA to maintain the ratio.

Summary of main findings.

1. Basal concentrations of PNA have pro-inflammatory effects when it stimulates tissues through their microcirculations.
2. In health, efferent sympathetic activity masks these effects.
3. The effect of PNA is overwhelmingly flow, not concentration, determined.
4. PNA stimulation has a neurodegenerative effect.

PART 2.

This part of the article explores the implications of the findings in part one .

The sources and sinks of PNA.

PNA overspills into the circulation from two sources. One, the dedicated nerve plexuses microcirculations have [32] and the other, the nerve plexuses of the tissues hosting microcirculations. The NNA of the plexuses constricts blood vessels while PNA dilates. It dilates arteries and veins by stimulating them by diffusion from their microcirculations and it dilates microcirculations by a flow dependent intraluminal effect [24] [33]. PNA is cleared from the blood by uptake into the nerves and mast cells, by monoamine oxidase inactivation and pulmonary clearance, the evidence of the latter being the lower concentration of PNA in arterial as compared to venous blood [34] and the acute increase in pro-inflammatory **markers** associated with pulmonary bypass procedures [35].

Vascular Tone**.**

Since PNA is believed to have no effect, vascular tone is attributed at present solely to the effect of NNA. Based on the findings in part one, vascular tone is in fact a resultant of PNA and NNA stimulation of a blood vessel. That is significant because it means when the effect of one stimulus is reduced the effect of the other is necessarily increased without there having been any increase in its concentration. Evidence to that effect is seen when vein grafts are harvested by the traditional method which involves resection of their adventitia with the accompanying loss of their microcirculations and PNA stimulation. As soon as that

resection occurs, ~95% of grafts go into spasm, due to the effective increase in the unopposed constrictor effect of NNA on the grafts. In contrast grafts harvested by a recently introduced 'no touch' technique that preserves their microcirculations rarely go into spasm [36], [37].

In addition to the increased tone, traditionally harvested grafts also display increased rigidity. The evidence of that is seen by the fact that surgeons sometimes have to perfuse grafts at pressures as high as 300 mm Hg, in addition to stimulating them with a cocktail of vasodilator drugs, to dilate and make them fit for purpose. The rigidity effect is not related to the ex vivo nature of grafts because the same effect occurs in the canine ascending aorta when its microcirculation is resected in vivo [38], and in arteries affected by Takayasu's Disease [39] and William's Syndrome [40] when their microcirculatory modules are blocked by inflammatory debris and the sections of the arteries serviced by those modules become stenosed and rigid. Rigidity is fact a function of the balance between the number of latchbridges created in non-skeletal muscle by the effect of NNA stimulation and the number of latchbridges released by the β_1 phosphorylating effect of PNA [41] responsible for relaxing non-skeletal muscle [42], [43].[44],[45].

The response to disturbance of the noradrenergic ratio.

When any factor disturbs the set point noradrenergic ratio of a tissue, the hypothalamic sympathetic centres are believed to be alerted to it by a change in the level of afferent sympathetic activity they receive from the affected tissue. The centres are then believed to become involved in restoring the ratio – in the process masking, but not eliminating, the changed effect of PNA - by adjusting the level of efferent sympathetic traffic it projects to the tissue. That masking effect is believed to be the reason why no response was detected

when NA was infused into healthy subjects until its concentration reached 1,800 pg/ml minimum [2]. As the concentration of infused NA increased the level of afferent sympathetic activity in the body is believed to have increased. In response to that the hypothalamic centres are believed to have increased the level of efferent sympathetic activity to the level where the infused NA had no detectable effect. However, because hypothalamic neurons have a limited ability to increase their level of activity [46], as the concentration of infused NA kept on rising and the level of afferent sympathetic activity it caused increased, the hypothalamic neurons eventually reached the limit of their ability to increase the level of efferent activity to the point required to maintain the set point noradrenergic ratio of tissues. At that point that ratio would be expected to shift in favour of PNA stimulation and cause a detectable effect. Based on the experimental findings, the point at that shift occurred was when the concentration of infused NA reached 1,800 pg/ml minimum [2].

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The credibility of this NAS paradigm rests on its ability to provide an internally coherent explanation of a number of findings that relate to the operation of the system and are difficult or impossible to account for at present. One is why infused ADR, unlike PNA, causes detectable effects at a low picogram concentration, the reason being ADR, unlike NA, doesn't evoke an opposing masking effect mediated by itself. Another is why an association exists between increased cardiac microcirculatory flow, increased cardiac afferent sympathetic activity and increased efferent sympathetic activity in dilator cardiac failure (v.i.) and, conversely, why there is an association between reduced cardiac microcirculatory flow and decreased cardiac efferent sympathetic activity in hypertrophic cardiomyopathy

[47]. In the latter case the reason is believed to be that reduced cardiac PNA stimulation shifts the noradrenergic ratio of the heart in favour of the growth promoting, anti-inflammatory effect of NNA stimulation on the heart, hence the cardiac hypertrophy effect. That same shift in favour of NNA stimulation and hypertrophy occurs in preeclampsia [48] where a factor that decreases perfusion of the uterus shifts the noradrenergic ratio of that organ in favour of the growth promoting effect of NNA and causes it to hypertrophy [49]. Finally, the paradigm accounts for the fact that, in life, the level of efferent sympathetic activity in the body increases in line with the genetically programmed rise in the concentration of PNA [50]. The increased neurotoxic effect of this programmed rise in PNA concentration makes it impossible for the NAS to continue stabilising the PNA/NNA ratio at the level characteristic of youth and that, I speculate, is responsible for driving the ageing process.

The effect of cholesterol plaques on the noradrenergic ratio.

Cholesterol plaques are the commonest cause of increasing the flow dependent effect of PNA and, thus, of shifting the noradrenergic ratio of a tissue in favour of PNA. They have that effect because of the distributive haemodynamic effect of the turbulencethey induce in arteries through the roughness of their surfaces and their protrusion into the arterial lumen (v.i). Because plaques progressively increase in number and size, they progressivly increase microcirculatory flow and the pro inflammatory effects of PNA in arteriosclerosis, of which, crucially, a degenerative neurotoxic effect is one.. Consistent with arteriosclerosis being associated with a neurotoxic effect, when rabbits were made arteriosclerotic by diet the nerves of their pulmonary and aortic arteries eventually ceased to respond to electrical

stimulation, or to release [^{3}H] labelled NA 51]. Also consistent is the fact that cardiac neurotransmitter stores in dilator cardiac failure are sometimes only 10% of normal [52].

The sensitivity of the hypothalamic sympathetic centres.

The hypothalamic sympathetic centres are highly sensitive to changes in the level of afferent sympathetic activity they detect coming from tissues affected by changes in PNA stimulation and equally precise in their responses to any change they detect. Those conclusions are drawn from the fact that every heart beat is associated with a spike of efferent sympathetic activity in the nerves of the skin and muscle [53] and the of the kidney [54]**.** Those spikes, that are unrelated to baroreceptor reflexes [53], are believed to be responses by the hypothalamic centres to the momentary increase in afferent sympathetic activity they detect coming from the tissues following the momentary increase in the flow dependent effect of PNA all tissues experience with every heart beat.

An appreciation of the high priority the NAS gives to maintaining its set point ratio can be gained from the fact that during exercise, when one might anticipate efferent sympathetic activity to the microcirculations of muscles would be reduced, in order to increase blood flow, is, in fact, increased [55].

The obligatory role of lateral inhibition (contrast) in the noradrenergic system.

The NAS is designed to transmit regulatory information to the adrenergically innervated tissues of the body and to do that it displays the three features every system transmitting information displays viz., (1) a pattern that encrypts the information to be transmitted and (2) a feature that provides contrast, of (3) an appropriate level, to the pattern [56],[57]. The failure of any of those features to function appropriately distorts the information a pattern

is intended to transmit to its target sensor. Bar codes illustrate that principle well. The pattern of lines in a bar code encodes the information it is designed to transmit and the colour of the surface on which the pattern is printed provides the contrast needed to detect and decode the information the barcode encrypts. If the level of contrast between lines and the surface on which it is printed is too great or too little it becomes progressively more difficult detect and decode the bar code pattern. Lateral inhibition is the technical term used to connote the contrast that sharpens the details of signals encrypted in biological patterns and it is a feature that has been detected in every biological system receiving or transferring information in the body investigated to date [56,] [58]. The importance of lateral inhibition is demonstrated by it being recognised as the most potent physiological modulator of the excitor component of a system transmitting information [57]. The two contrasting patterns of NA involved in transmission of regulatory information are generated by NA diffusing from two different sources [58], the adrenergic synapses/boutons in the case of NNA and the porous postcapillaries of microcirculations in the case of PNA. Diffusion patterns are defined by the ratios between their component fluxes, with the polarity of the major flux determining the polarity [59] and, by extension, the effect of a pattern. The polarities of the NNA and PNA are speculated to be in planes at right angles to one another [13],[42]. Once generated, NA patterns are transformed and propagated throughout a tissue by second messengers passing through gap junctions that amplify them and accelerate their rate of propagation. The classic example of that type of transformation and propagation is the spread of the excitor pattern of NNA stimulation throughout the myocardium by the gap junction rich Bundle of His.

Equivalence between non-neuronal NA in the periphery and the brain?

The peripheral NAS is believed to have a close functional counterpart operating within the brain [61]. There, what has been termed non-synaptic NA [62] is believed to overspill from the brain into its cavities before circulating in the CSF over the brain surface where, I speculate, it diffuses back into the brain through the fine pores detected at the bases of the cerebral gyri and where the diffusion pattern generated by this diffusion process then provides the contrast brain cells need to detect and respond to the regulatory information encrypted in the diffusion pattern of synaptic NA. The existence of this possible functional equivalence between the effects of microcirculatory PNA and non-synaptic NA has the potential for regulatory confusion were the two forms of NA to mix, and to prevent that happening may be the reason for the existence of the blood/brain and the blood/CSF barriers to NA. Such functional equivalence would also be consistent with the fact that an inflammatory brain condition, like Parkinson's, is associated with increased CSF flow [63] and neuro-degeneration [64].

PART 3.

This is devoted to analysing the aetiologies of various clinical diseases in the light of the fresh concepts described above. Because the analysis is so dependent on microcirculatory flow I believe a basic knowledge of the morphology of microcirculations is essential for its understanding.

The morphology of microcirculations.

Microcirculations are networks of blood vessels ranging in size from arterioles to pulmonary arteries. They are constricted by the NNA released from their dedicated adrenergic networks [32] and are dilated by the PNA perfusing their lumens. Their role is to distribute blood channelled to the tissues by the arteries, veins and right heart and drained from the tissues by two interconnected systems, whose existence is visibly exemplified by the venae comites in skeletal muscle and the twin pulmonary veins in the lungs. Except for the pulmonary circulation, drainage is preferentially into the caval system but if that is not possible it takes place into an unvalved network that interconnects with every other microcirculation [17] and drains into the vena cava through the azygos system of veins [65] [66]. Because microcirculations have a single supply and a dual drainage system, they display the iconic feature of vascular triads, commonly featuring as an arterial vessel flanked by twin venous vessels. All microcirculations appear to be modular in construction, with each module of possessing an A/V shunt that makes it possible, by diverting its arterial inflow, for a microcirculation to be perfused by reflux through either of its twin drainage systems. The density of arterial microcirculations increases to the extent their hosts are affected by arteriosclerosis [67],[68],[69].

The primary role of microcirculations.

Since their discovery in the 18th century, the primary role of microcirculations has been and still is believed to be nutritional and metabolic. However doubts about that have existed since 1876, at least, when Köester observed that many small veins had more vasa than their nutritional needs seemed to justify [70]. Köester's doubts have been shown to be fully justified. Three morphometric studies of the microcirculations of various arteries and veins, in the periphery and the brain [71],[72],[73], have found no correlation between the density of a microcirculation and the apparent nutritional needs of its host. That and the evidence that the capsules of the kidney (v.i.) and liver [46], contain microcirculations that have no significant nutritional or metabolic role vis-à-vis their hosts, and the alleged presence of typical microcirculations on 3-5 cell thin aortas of young mice, all support the belief that the primary purpose of a microcirculation is to provide its host with the level of PNA stimulation it requires to maintain its set point noradrenergic ratio. In the circumstance, the nutritional and metabolic roles some microcirculations serve are seen as being secondary and opportunistic.

Factors affecting microcirculatory flow.

In terms of its significance turbulence may be considered the most important factor altering flow in a microcirculation. If no turbulence existed the absolute axial velocity vector of laminar flow would make it impossible for blood to leave an artery and perfuse a microcirculation. By its effect of introducing radial components into the velocity vector of blood turbulence pumps volume, content and momentum [74] from an artery into its primary branches and from them into its second order branches supplying its

microcirculatory modules and its third order branches supplying the microcirculatory modules of its companion veins [13].

Turbulence is induced in every artery at the free margins of its primary branch points by the boundary layer of the bloodstream reducing contact with the arterial endothelium and releasing some of the shear stress energy it has accumulated. When released, the energy is conserved and converted into turbulence [75] that migrates into the primary branches of the artery and reaches its peak distributive effect about 2-3 branch diameters downstream [76] [77]. There, the openings of the second order branches dedicated to supplying the arterial and venous microcirculatory modules are located [78] [79]. The intensity of the turbulence at those second order branch points determines the volume of blood that, under prevailing conditions, perfuses arterial and venous microcirculatory modules.

A number of factors stabilise the microcirculatory flow volume and, by extension, the noradrenergic set point ratio of a tissue. A stable cardiac output, a stable subdivision of the output and baroreceptor reflexes are major upstream factors; a stable level of shear stress between the endothelium and the boundary layer of the bloodstream is a major downstream factor. Such stability results in stable intensities of turbulence at branch points, and in stable volumes of blood leaving arteries and perfusing microcirculatory modules and generating stable levels of PNA stimulation. The level of shear stress between the blood stream and the endothelium is micro managed by the ability of the endothelial cells to detect the level of longitudinal stress between them and the blood stream [80] and to respond to any deviation from the designed level it detects by releasing a factor, like NO, that dilates the artery, thereby lowering the stress level, or, that like endothelin, constricts the artery and increases the stress level. Extrinsic factors like cholesterol plaques, accessory

cervical ribs, stenoses or aneurysms induce pathological turbulence. Depending on the intensity of that turbulence those features can shift the noradrenergic ratio of an artery in favour of the inflammatory effects of PNA, of which one is believed to be a reduced bioavailability of endothelial NO [81]. Consistent with that belief, the $beta_1$ blocker nebivolol, by inhibiting the β_1 agonist effect of microcirculatory PNA, increases NO bioavailability [82]. Because reducing NO bioavailability increases the level of shear stress between the endothelium and the blood stream it increases the intensity of the turbulence induced at branch points and, consequently, it increases the volume of microcirculatory flow. That implies the increased inhibitory effect of PNA stimulation on the endothelium initiates a positive feedback that progressively reduces NO bioavailability, that has the effect of progressively increasing microcirculatory flow and the pro inflammatory effect of PNA.

Plaques and microcirculatory bloodflow.

Using monkeys made arteriosclerotic by high fat diets, Heistad and his colleagues have demonstrated that cholesterol plaques are associated with increased microcirculatory flow, independent of any change in blood pressure, in the monkeys' aortic and coronary microcirculations. Presumably the turbulence induced by the plaques is responsible for the increase. In one experiment plaques were associated with an increase in flow in the microcirculations of thoracic aortas of from 1.3 to 17ml/min/100gm and in the microcirculations of abdominal aortas of from 2.2 to 31ml/min/100gm [83]; in another, plaques were associated with an increase in flow in the microcirculations of coronary arteries of from 3 to 16ml/100gm in one case [84] and 5 to 47 ml/100gm in another [85]. Interestingly, in the latter experiment, when the monkeys' diets were returned to normal

the plaques receded and the changes in microcirculatory flow reversed spontaneously, again independently of any change in blood pressure.

The cardiac microcirculation.

With one exception, the same principles govern the operation of the cardiac microcirculation as govern those of arteries in general. So, the cardiac microcirculation displays a modular construction, each one servicing a single cardiac chamber, and vascular triads that characterise every microcirculation [80]. Similarly, the volume of blood perfusing the cardiac microcirculation is determined, under prevailing conditions, by the intensity of the turbulence induced at the root of the aorta [86] when blood is ejected from the left ventricle and reduces contact with the edges of the aortic valve, and, by migration, creates turbulence close to the origins of the coronary arteries [87], the topological equivalents of the second order branches of the heart.

The major difference between the cardiac and arterial microcirculations is that flow and the effect of PNA stimulation is intermittent in the heart, occurring only during diastole. The converse is true of NNA stimulation which occurs only in systole. The effect of that dichotomy is that NNA and PNA stimulate the heart in isolation and consequently, NNA contracts and PNA relaxes the heart to the maximum of both their potentials – an ideal situation in an organ designed to act as a two stroke pump. By analogy with what happens in vein grafts harvested in the traditionally manner (v.s.), the effect of unopposed cardiac NNA stimulation would be the creation of the maximum possible number of latchbridges within the myocardial syncytium, thereby making the heart more rigid during systole , as has been observed [80]. Another effect that would be expected to occur during systole, and is

believed to occur [86], is a decrease in the diameters of the arterioles and the venules of the cardiac microcirculation caused by the loss of the intraluminal dilator effect of PNA.

At the end of systole, flow returns to normal rapidly in the cardiac microcirculation. That causes an equally rapid increase in the flow dependent effect of PNA stimulation on the heart, exaggerated by being unopposed by the effect of NNA stimulation. That has the effect of cardiac PNA stimulation releasing the maximum possible number of the latchbridges created during systole by NNA stimulation and of relaxing the heart to the greatest possible extent possible, under the circumstances, during diastole, thus preparing it to the greatest extent possible, under prevailing circumstances, for the following episode of systole. The evidence that the heart needs to be stimulated by PNA if it is to relax can be seen by the fact that when flow is blocked anywhere in the cardiac microcirculation the local myocardium goes into sustained spasm due to the latchbridges created by NNA stimulation during the prior episode of systole being maintained. Another effect PNA stimulation would be expected to have during diastole, and that it appears to have [80], is an increase in the diameters of the venules and arterioles of the cardiac microcirculation caused by the intraluminal dilator effect of PNA, in the absence of the constrictor effect of NNA.

Arteriosclerotic dilator cardiac failure.

Arteriosclerotic dilator cardiac failure is an inflammatory condition characterised by an increased level of efferent sympathetic activity, that is reflected in an increased level of NA overspill in the blood draining from the heart. Normally that level is ~10 ng/min, but in dilator failure it can go as high as 131 ng [89]. That increased efferent activity, in turn, is believed to be evoked by the effect of the increased afferent activity associated with heart

failure [90] that is caused by the increased cardiac microcirculatory flow, caused by the pathological turbulence induced at the root of the aorta and in the coronary sinuses by the cholesterol plaques and calcifications located on the free edges and the surfaces of the aortic valves. The evidence of that increased flow is masked by the increased cardiac efferent activity and by two features that reduce the fraction of the flow that reaches the postcapillary networks of the heart's microcirculation where PNA causes its stimulatory effect. The features in question are shunts from the coronaries to the microcirculation of the pulmonary artery [91] and the development of a pathological microcirculation on the coronary epicardials. The flow of blood into that pathological microcirculation reduces the effect of the increased coronary flow in two ways, one, by the simple fact of blood diversion from the coronaries into the pathological microcirculation and, two, by the fact that when the PNA in the diverted blood diffuses from the pathological microcirculation it constricts the coronaries, thus impeding coronary flow. If the constrictor effect is sufficiently severe, as it may be when coronary flow increases during effort, it causes angina pectoris that, pharmacologically, but not physiologically [20], is paradoxically responsive to the β_1 agonist effect of beta blockers. This "paradoxical" constrictor effect is readily duplicated experimentally, using ACh and serotonin [92] [93] instead of NA. One explanation for this "paradoxical" effect could be that when PNA diffuses from the pathological microcirculation and stimulates the epicardials it converts from being an intraluminal dilator of the pathological microcirculation to being a constrictor of the epicardials. In doing that the PNA would be merely copying the conversion effect ISO and NA display in the CLSV segment where ISO dilates and NA constricts the segment when they act on it intraluminally but ISO constricts and NA dilates the segment when they stimulate it by diffusion from its microcirculation. Consistent with that explanation, beta blockers have what is presently

considered to be a paradoxical therapeutic effect on angina [94]. Reinforcing the credibility of this explanation is the fact that the lungs have a comparable, though normal, arrangement as the heart in terms of having one microcirculation hosting another and of being associated with angina of effort in cases of pulmonary hypertension.

Pulmonary hypertension.

The two microcirculations in the lungs consist of the pulmonary artery and its branches and the microcirculation of those vessels [89][92]. Unlike every other microcirculation, the microcirculation of the pulmonary artery drains into its host rather than is supplied by it [91]. Consequently, when any lesion, plexiform or other, impedes flow in the pulmonary artery and its branches it impedes flow in its microcirculation and that impedance, if significant, causes blood in the artery to be shunted into its microcirculation and from there into the vena cava through the coronary veins, thereby creating a satellite circulation in the process that imposes an added burden on the right heart. And just as when flow increases beyond a critical level in the pathological microcirculation of the coronary epicardials and causes angina pectoris, when impedance in the pulmonary artery causes a critical increase level of reflux in its microcirculation, that can be expected to cause pulmonary angina, due in this instance to PNA in the pulmonary microcirculation causing constriction of the pulmonary artery. Consistent with that stimulating effect of PNA, the endothelial cells of the pulmonary artery display a reduced NO bioavailability [95].

The neurotoxic effect of increased PNA stimulation.

Given the evidence of the degenerative effect increased PNA stimulation has on the adrenergic nerves of varicosities [32] and the evidence that the adrenergic nerves of the pulmonary and aortic arteries of rabbits with diet induced arteriosclerosis soon fail to respond to electrical stimulation [51], it is not unexpected that the adrenergic nerves of failing arteriosclerotic hearts display evidence of progressive damage in that condition [52]. Because of that the cardiac nerves in dilator failure eventually reach the stage where they can no longer respond fully to the increased level of efferent sympathetic activity they receive from the hypothalamus . That predictably results in the NAS being no longer capable of maintaining the heart's set point noradrenergic ratio, which progressively shifts in favour of the degenerative effect of increased PNA stimulation. Evidence of the damage caused by by that increased PNA stimulation includes depletion of their neurotransmitter stores to as little as 10% of normal, sometimes, and a drastic reduction in the quantity of neurotransmitter released per nerve impulse [96]. Other critical consequences of the shift in favour of cardiac PNA stimulation include an increased distensibility of the heart [97], resulting from a shift in favour of latchbridge release as against latchbridge formation in the myocardial syncytium, a lengthening of the cardiomyocyte sarcomeres to a suboptimal 2.4-2.5 µm [96] and a remodeling of the heart from an asymmetrical prolate ellipse shape to a more pliable, less efficient spherical shape [98].

Hypertrophic Cardiomyopathy.

Hypertrophic cardiomyopathy is a non-inflammatory genetic condition, affecting all ages, associated with mutations encoding the sarcomeric proteins. In terms of blood supply and PNA stimulation, hypertrophied hearts are characterised by features that either cause or

reflect a tendency towards cardiac ischaemia. Those features include coronary arteries with thickened walls and narrowed lumens [99], evidence of decreased coronary flow [100], [101], evidence of impaired myocardial blood flow during stress [102] and of an inadequate increase in myocardial blood flow in response to dypyridamole infusion [103]. All those features either actually or tend to reduce cardiac microcirculatory flow volume and shift the cardiac noradrenergic ratio in favour of the growth promoting effect of NNA stimulation, thus causing the heart to hypertrophy. That same hypertrophy effect occurs in preeclampsia when the uterus experiences a reduction in blood flow and becomes hypertrophied [49]. Consistent with cardiac hypertrophy not being an inflammatory condition, beta blockers do not modify the course of the condition [104] and the condition is characterised by a reduction in efferent cardiac sympathetic activity [105].

Renal Hypertension.

A kidney possesses three networks of fine blood vessels, of which two are well known, the glomerular and the peritubular. The third, little known, is located in the capsule of the kidney (Fig 6). The first two networks are not microcirculations in the technical sense. Their role is purely executive, maintaining the salt and water balance of the body under regulation by the renal nerves and intrinsic autoregulatory mechanisms. The fine network of vessels in the renal capsule is a true microcirculation, as evidenced by the presence of vascular triads (fig 6) and A/V shunts that appear to open into the subcapsular stellate veins.

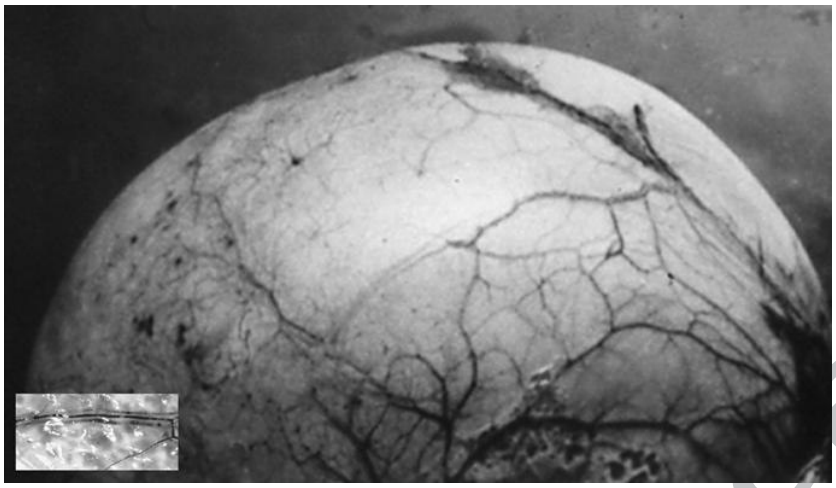

FIG 6

Photograph of the renal microcirculation located in the capsule of a kidney with (inset) a micrograph of a section of a vascular triad.

The renal capsular microcirculation (RCM) receives supply from the renal and lobar arteries but it is functionally independent of those sources, as evidenced by the fact that it continues to be perfused when the renal artery is thrombosed or the kidney is avulsed [106]. As its size and peripheral location would indicate the RCM has no metabolic or nutritional role in relation to the renal parenchyma, they being adequately provided for by the periglomerular and peritubular networks. The role of the RCM seems to be purely regulatory, providing the kidney with the PNA stimulation it needs to maintain its set point noradrenergic ratio.

Possibly reflecting the multi lobular development of the kidney, the RCM is exceptional in being supplied by several known named arteries, including the aorta [61], renal, lobar, subdiaphragmatic and adrenal [107] [108]. Given that the basic cardiac output is constant, except in pregnancy, whenever blood flow is impeded anywhere in the body or a

capacitance is reduced, blood is necessarily displaced to other parts of the circulation. And because the RCM has so many inflows, it can be expected to receive a disproportionate fraction of any displaced blood. That, in turn, implies when any factor blocks blood flow anywhere in the body that it would lead to a disproportionate increase in renal afferent sympathetic activity and evoke a disproportionate increase in renal efferent sympathetic activity, that would cause a disproportionate increase in JGA stimulation and hypertension, presumably designed to restore the normal blood flow pattern.

Consistent with this analysis, a reduction in splanchnic capacitance [109], a reduction in cerebral blood flow caused by fibroplasia of the carotids and vertebral arteries [110], vascular fibroplasias in general [111], compression of the aorta distal to the renal artery in preeclampsia, causing a minimum 40-50% reduction in blood flow distal to the compression [112], the obstruction of multiple microcirculatory modules in Takayasu's arteritis [39], Williams Syndrome [40] and polyarteritis nodosa [111], the roughening of the aorta by cholesterol plaques, causing pathological turbulence that increases inflow to the many different vessels originating in the aorta and supplying the RCM, are all conditions that cause blood to be preferentially displaced into the renal microcirculation and are known to be associated with hypertension. In addition to this list, the increased microcirculatory flow associated with polycythemia [113] is associated with hypertension [114].

Because each renal artery receives about 10% of the total cardiac output, any significant impedance to flow in that vessel is particularly likely, because its origin is so close to the origins of the arteries supplying the renal microcirculation, to result in a significant redistribution of blood to that microcirculation and cause hypertension. Consistent with that analysis any obstruction that reduces renal blood flow by about 60-70% is commonly

associated with hypertension [111]. In the circumstances, it is understandable why Goldblatt caused hypertension when he placed a clip on a renal artery.

That the renal capsule has a factor responsible for causing hypertension has been known for more than a century. That was discovered by chance in the late 19th century when, as a final resort, some European physicians empirically decapsulated the kidneys of patients with terminal renal disease and preeclamptic hypertension and found it had a therapeutic effect in both conditions [115] [116] [117]. However, because of the morbidity and mortality associated with the procedure it was abandoned around the middle of the last century. Nowadays decapsulation is purely an experimental procedure that when employed is associated with significant changes in kidney function [118].

Consistent with increased renal PNA stimulation being the cause of hypertension that condition is a recognised inflammatory disorder [119]. And consistent with that beta blockers and renal afferent nerve ablation have a significant therapeutic effect on the condition [120].

Preeclampsia.

Preeclampsia is unique in being associated with two contrasting shifts in the noradrenergic ratio. In the case of the mother's tissues in general the shift is in favour of PNA; in her uterus and conceptus it is believed to be in favour of NNA [48]. Consistent with that dichotomy, one investigator has claimed preeclampsia has a heterogenous cause [123]. The contrasting shifts in ratios in preeclampsia are believed to result from the pregnant uterus becoming retroflexed and compressing the aorta against the lordotic curve of the spine [122] or the brim of the pelvis [48] with the result, in both cases, of reducing blood flow

beyond the compression by a minimum 40-50 % [123] and causing ischaemia of the uterus and the conceptus which are supplied by arteries originating distal to the compression. Given the stability of the cardiac output referred to earlier and the 30% gestational increase in blood volume associated with pregnancy, that exceptionally large percentage reduction in blood flow beyond the aortic compression is necessarily associated with an exceptionally large diversion of blood flow to the microcirculations of the arteries originating upstream of the compression. Given the RCM receives a disproportionally large fraction of any diverted flow, hypertension is one predictable feature of preeclampsia. Other predictable and observed features of preeclampsia include an increase in renal efferent sympathetic activity [121] and a tendency to develop left ventricular failure due to increased flow in the cardiac microcirculation [124].

Because of the ischaemia they experience in preeclampsia, the uterus and the conceptus experience a decreased level of PNA stimulation and, by default, a relatively increased level of NNA stimulation. And just as the heart hypertrophies when it experiences an equivalent shift in hypertrophic cardiopathy, the uterus hypertrophies in preeclampsia [49]. LIkewise, just as the vessels of a microcirculation constrict when they experience a decrease in the dilator effect of intraluminal PNA stimulation, the spiral uterine arteries, which represent the microcirculation of the uterus, also display a reduction in diameter, with consequent poor placental development, in preeclampsia. Moreover, because the child in the uterus experiences a reduction in PNA stimulation in preeclampsia, the use of beta blockers for the treatment of the maternal hypertension in preeclampsia is contraindicated, because their use reduces still further the level of PNA stimulation the child needs to maintain its proper PNA/NNA ratio and causes it respiratory depression [125] [126]. Finally, the fact

preeclampsia involves two contrasting pathologies, any drug therapy designed to benefit one is likely to aggravate the other. That is the reason, I believe, why modern therapeutic advances have done so little to improve the outcome for patients with preeclampsia compared to what they have done to improve the outcome for patients suffering from other serious conditions like cardiac failure and hypertension.

DISCUSSION

Because the findings on which the hypothesis and the paradigm of the NAS presented in this paper are based have not been independently verified, nor can they be experimentally duplicated in vivo in a healthy subject, they cannot be validated in the normal way. However, on the grounds of what they have led to the findings deserve a certain credibility. These grounds include the creation of a paradigm of the NAS that can account for several features associated with the operation of the sympathetic system that up to now have not been satisfactorily explained and the generation of a hypothesis that provides a unified, internally consistent explanation for a variety of inflammatory and non inflammatory disorders, affecting different tissues, and that rationalises the evolutionary conservation of PNA, the existence of valve aggers and the blood/brain and blood/ CSF barriers to NA. Further adding to the credibility of the findings is the fact they can resolve a number of pharmacological anomalies, such as the paradoxical use of beta blockers to treat angina and hypertension and provide an explanation for the long questioned [127] intriguing claim by Furchgott and Zawadski [128] that endothelial cells are obligatory for low concentrations of ACh to relax vascular smooth muscle cells. That claim resulted from an experiment, independently famous for having led to the discovery of NO, where Furchgott and his colleague abraded the endothelium of an aortic strip and discovered the strip no longer relaxed in response to stimulation by low concentrations of ACh because, they contended, endothelial cells had a factor that relaxed vascular smooth muscle but that required stimulation by ACh to be activated. The validity of that claim has been disputed for years [127] on the grounds that it is difficult to conceive why endothelial cells would have

muscarinic receptors to enable intraluminal ACh activate a factor obligatory for the relaxation of vascular smooth muscle when plasma and red blood cells contain a range of specific and non specific esterases to ensure they would never experience stimulation by ACh. A review of Furchgott's paper reveals he had failed to appreciate the greater sensitivity of vascular smooth muscle to drugs stimulating it by diffusion from a vascular microcirculation than intraluminally. So, when Furchgott reduced the concentration of ACh below the threshold at which it contracted the aortic strip acting intraluminally, the drug still retained the ability to relax it by stimulating it by diffusion from its microcirculation. Believing an endothelial factor had to have been responsible for this relaxation effect, Furchgott abraded the endothelium and found the low concentration drug no longer relaxed the strip, thus apparently confirming the validity of his hypothesis. However, as evident from a photograph in Furchgott's paper, it is more likely ACh failed to relax the strip because the abraded endothelial cells had plugged the primary branch openings of the strip and, thus, prevented ACh from stimulating the strip through its microcirculation and relaxing it, in the same way as an injection of 50µM Ach relaxed the canine tibial vein when it stimulated it by diffusion from its microcirculation, after having been injected into the lumen of the cranial tibial artery [13].

Adding credibility to the hypothesis presented that, in essence, claims health depends on a balance between flow dependent effect of PNA and NNA is the fact that that claim resonates, to a greater or lesser extent, with several other long recognised claims. One, credited to Recklinghausen [129], goes back to 1883, and claims that changes in microcirculatory flow cause remodeling changes in arteries. Another claim goes back much further, to beyond the time of Galen, and claims that bleeding patients has a therapeutic

effect. That it indeed may have would seem to be borne out by the fact that the procedure remained popular right up to the time of William Osler, "The Father of Modern Medicine", who died in 1919 and who allegedly used it on occasions. The present hypothesis would rationalise any therapeutic success of bleeding by claiming that bleeding necessarily reduces microcirculatory flow and, with it, the flow dependent, malign effect of PNA stimulation. Two further beliefs, with a more tenous resonance to the present hypothesis, include Hippocrates' credo that 'like cures like' and the oriental notion that health depends on the maintenance of a balance between the negative effects of Yin and the positive effects of Yang.

If increased PNA stimulation has the degenerative and ageing effects credited to it in this article then the concept of introducing low dose beta blockade at an age when people are still apparently healthy should be considered. Or, alternatively, it might be worth determining the value of an individual's noradrenergic ratio at around thirty, when he/she is healthiest, and then attempting to maintain that value long term by the use of an appropriate combination of alpha and beta blocker drugs. Or, assuming the rationalisation offered for the therapeutic benefit of bleeding is valid, then it might be worth exploring the possibility of developing a less dangerous method than bleeding for reducing the overall levels of PNA stimulation in patients with inflammatory conditions. That could involve the development of an extracorporeal means of dialysing a patient's PNA or the use of exchange transfusion of a patient's own stored blood, previously dialysed free of PNA. The belief that those procedures would have a worthwhile therapeutic effect is consistent with the rejuvenating effect of heterochronic parabiosis. That is a classical experimental procedure where the circulations of an elderly and a young animal are joined and within a few months

the tissues and cognitive abilities of the elderly animal appear to be rejuvenated [130]. Based on the hypothesis presented here that rejuvenation effect would be explained as having been caused by the noradrenergic system of the vigorous young animal decreasing the concentration of PNA in the common circulatory pool to the level appropriate to young animals, which is significantly lower than that in elderly animals. That would be expected to have the effect of shifting the balance between the stimulatory effects of PNA and NNA in the elderly animal in favour of the anti-inflammatory, growth promoting effects of NNA.

Finally, this paper has highlighted what is believed to be the crucial role of cholesterol plaques in increasing the damaging flow dependent effects of PNA. That should encourage those working in the relevant fields of molecular biology to redouble efforts to develop a vaccine or other means of delaying the appearance or reducing the size and number of plaques. It should also influence those trying to persuade the public of the importance of diet in reducing plaque formation and heart disease to target their message at a younger age group then they do at present.

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