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MECHANISMS OF CATECHOLAMINE RELEASE

FROM THE ADRENAL MEDULLA

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The adrenal medulla is often described as an enlarged sympathetic ganglion. Its chromaffin cells, the analogues of the post ganglionic neurones, release the neurotransmitter, adrenaline, into the bloodstream in harmony with a generalised increase in sympathetic nervous system activity such as occurs in response to stress.

However, I propose to put forward the view, supported by research done with Dr. Alexander Ungar, that the regulation of adrenal medullary secretion is far more complex. Not only is the neural control much more selective than usually considered but also catecholamine release is modulated by the surrounding adrenal cortex.

One of the earliest descriptions of the adrenal glands was made by Eustachius in 1563 and later Bartholinus (1611) attributed to them the secretion of the "black bile or atrabile". Winslow named them the "suprarenal glands". Although this is an appropriate term for erect primates, adrenal is more suitable as they are separate from but near to the kidneys in other species. The mammalian adrenal gland consists of an outer cortex and an inner medulla which are enclosed within the capsule of the gland. The cortex, synthesising steroid hormones, is under humoral control while the medulla has a preganglionic sympathetic innervation and releases the catecholamines, noradrenaline and the methylated derivative, adrenaline.

The proportion of these two adrenal catecholamines varies with species and the human gland contains predominantly adrenaline while dogs and cats have more equal amounts. However, noradrenaline was not considered to be an adrenal catecholamine until 1949 and thus the early work on the releasing stimuli was only with reference to adrenaline.

This was the situation when Walter B. Cannon began to formulate his ideas on the emergency role of the adrenal medulla in stress and put forward the concept of the sympathetic nervous system as the "flight and fight" mechanism. A single adrenal hormone fitted in well with his view that the sympathetic exerted a diffuse and general influence over the structures which it innervated. He contrasted the sympatho-adrenal system with the parasympathetic which, unlike the former system, dealt with organs separately and not as a group. Cannon described sympatho-adrenal activity evoking widespread changes, such as dilating pupils, accelerating the pulse and constricting splanchnic blood vessels while a reduction in its activity permitted these widely scattered organs to resume their former state. "Because of their double innervation the organs under tonic control of the autonomic system can be made to alter the degree of their functional use either as an integrated group, through the sympatho-adrenal system or as separate structures through the direct effects of parasympathetic impulses" (Cannon, 1930's).

Cannon's flight and fight concept profoundly influenced this area of biological thought and although it is

a useful generalisation, it has hampered the understanding of the ability of the sympathetic nervous system to respond selectively. There is good evidence that in a number of reflex responses, the sympathetic drive to one organ increases while that to another decreases and is thus not acting as the diffuse unselective system which Cannon proposed. For example, the primary reflex from the arterial chemoreceptors as well as stimulating respiration results in peripheral systemic vasoconstriction with a withdrawal of cardiac sympathetic drive. However, the increased ventilation invokes the lung stretch reflex and this reduces vasoconstrictor tone while at the same time increasing sympathetic drive to the heart. (Daly and Robinson, 1968)

We wondered whether the nervous system could selectively control adrenaline and noradrenaline secretion from the adrenal medulla or were the two catecholamines always released in the same proportions. Studies in man were out of the question for adrenal venous sampling is essential as the noradrenaline in peripheral blood samples predominantly originates from adrenergic neurones rather than the adrenal medulla. Thus most experiments had been performed on dogs or cats. There was considerable controversy as to whether independent control of the two catecholamines' release occurred.

In France, Malmejac and his associates had used dogs and from numerous publications concluded that noradrenaline and adrenaline were always released in the same proportions from the adrenal gland.

However, the Scandinavian physiologists experimented on cats and U.S. von Euler was their main spokesman. He argued that various physiological stimuli could independently release noradrenaline or adrenaline from the adrenal medulla. He does not appear to have had a very high opinion of the French workers. In his book "Noradrenaline"(1956) he refers to only one of their publications and then only with regard to their results on resting secretion.

Malmejac and von Euler presented their opposing views at the XXI International Congress of Physiological Sciences in Buenos Aires (1959) and there appears to have been much debate. Although Malmejac wrote a Physiological Review in 1964, his team's work has been largely ignored. This was, in part, due to von Euler's prestige but Malmejac's case has not been helped by his habit of publishing work in the form of numerous small and repetitive abstracts in a relatively inaccessible French journal, Comptes Rendus de la Société Biologique.

Over the past twenty five years various authors have argued for and against selective adrenal catecholamine release but in all that I have read, no one has made more than a passing reference to the possibility of there being a species difference between the dog and the cat. Although many writers had discussed a great deal of the work to support one or other view, no one had tried to explain the controversy by examining the published data to see if the differences were related to species. Furthermore, no one laboratory had given the same set of reflex stimuli to both dogs and cats.



On examining the literature I found that in the dog the data suggested that whatever the nature of the stimulus to the adrenal gland, noradrenaline and adrenaline are released in about the ratio of 1 to 4. Any claims for independent secretion of one catecholamine (-it was always adrenaline) were based on comparisons with the proportions in very low resting outputs and one finds that the 1 to 4 ratio held for the composition of the releases.

However, all those workers who examined adrenal catecholamine release in cats found evidence of selective release. Carotid occlusion and haemorrhage, which exert part of their effect on the adrenal medulla via the baroreceptor reflex, give rise to a secretion containing about 70% noradrenaline (Kaindl and von Euler (1951), von Euler and Folkow (1953) and Fuerstein and Gutman (1971)). There is good evidence that hypoglycaemia releases predominantly adrenaline (Dunér (1954), Fuerstein and Gutman (1971)) and from Dunér's work it appears that blood glucose exerts its reflex effects only on adrenaline secretion. Asphyxia and cyanide-induced chemoreceptor stimulation seem to preferentially release adrenaline (Redgate and Gellhorn (1953) and Anichkov et al (1960)) but this work depended on less reliable assays. Stimulation in the region of the hypothalamus has been shown by three groups to release adrenaline selectively (Redgate and Gellhorn (1953), Folkow and von Euler (1954) and Grant et al (1958)). Folkow and von Euler (1954) also found stimulation points in the brain which selectively released adrenaline or varying proportions of the two catecholamines.

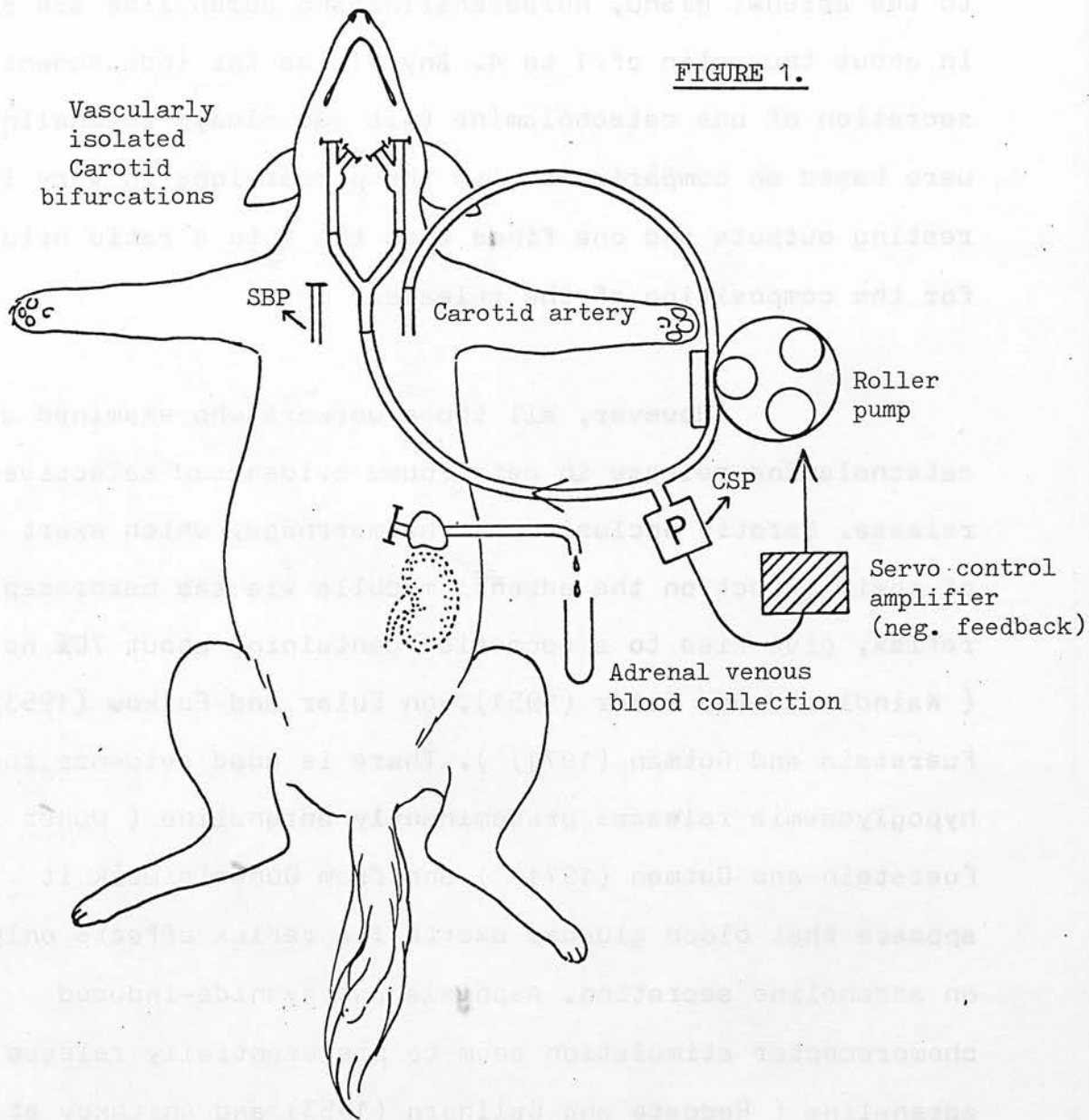


FIGURE 1.

CAROTID BIFURCATION PERFUSION AND ADRENAL VENOUS BLOOD COLLECTION

These publications suggested that in cats the baroreceptor reflex releases predominantly noradrenaline while chemoreceptor stimulation releases adrenaline. However, stimuli like carotid occlusion are relatively non-specific having both baroreceptor and chemoreceptor components. Thus we designed an experimental technique for use in dogs and cats which would enable us to invoke independently the baroreceptor reflex from the carotid sinus stretch receptors and the chemoreceptor reflex from the carotid bodies. The animals were anaesthetised with chloralose-urethane or sodium pentobarbitone.

The main arterial branches of the carotid sinus regions on both sides were ligated leaving the lingual arteries open as an outflow which does not have significant anastomosis with the brain. The vascularly isolated carotid bifurcations were perfused with the same animal's arterial blood delivered at constant pressure by a roller pump. A servo-amplifier controlled the speed of the pump enabling the carotid sinus pressure to be set and maintained at will. The animals were artificially ventilated with air enriched with oxygen and the pH and  $P_{CO_2}$  of arterial blood were held at normal levels. The vagi were cut in the neck to remove the buffering effect of the aortic arch receptors. Baroreceptor reflexes were elicited by lowering the carotid sinus pressure from 150 to 80 mm Hg. The chemoreceptors were stimulated by lowering the  $P_{O_2}$  of the blood perfusing the carotid bifurcation.

The venous outflow of the left adrenal gland was collected for estimation of noradrenaline and adrenaline



by a spectrophotofluorimetric method. The percentages of noradrenaline in the gland's resting secretion and in that released by the baroreceptor and chemoreceptor reflexes were calculated. The total amounts of catecholamine in the incremental releases evoked by the two reflexes were of a similar size.

In the dogs we consistently found that the baroreceptor and chemoreceptor reflexes released the same proportions of noradrenaline and adrenaline as found in the adrenal gland's resting output. ( i.e. 20% noradrenaline ).

However, the situation was very different in the cats. The resting secretion contained about 45% noradrenaline while the incremental releases evoked by the baroreceptor and chemoreceptor reflexes contained 75% and 16% noradrenaline respectively. ( Critchley et al (1973,1980) ).

Our work explains the apparent controversy over the existence of selective adrenal catecholamine release. It is further evidence that the control of the sympathetic nervous system is much more complex than Cannon proposed. There is good evidence that noradrenaline and adrenaline are segregated into separate cells in the adrenal medulla ( Eranko 1958 ). To explain the selective release phenomenon, I can only presume that the relative proportions of the two types of cells innervated by the two "sympathetic" reflexes are different.

Using isolated Locke solution perfused adrenal glands, we demonstrated that both muscarinic and nicotinic agonist drugs release adrenal catecholamines. In cats but again not in dogs, we found that selective release occurred with muscarinic and nicotinic stimulation releasing predominantly adrenaline or noradrenaline respectively. ( Critchley et al, 1975 ). We found that in the intact animal nicotinic ganglionic blockade with hexamethonium selectively abolishes both the systemic vasoconstriction and adrenal catecholamine release in response to the baroreceptor reflex but not the chemoreceptor reflex. From our own research and that of others, it is beginning to appear that certain autonomic reflexes have discrete pathways in the sympathetic nervous system innervating organs selectively and passing through either predominantly muscarinic or nicotinic synapses.

However, to return to the adrenal medulla I have so far only considered neuronal mechanisms of catecholamine release. I would now like to discuss the evidence which led us to believe that there is an important humoral mechanism. Although it is generally accepted that the adrenal cortex and medulla function independently, there is growing evidence that the cortex does exert a significant influence on the medulla. The very phenomenon of their close association in mammals is strange as embryologically the medulla originates from neuroectoderm while the cortex is from coelomic epithelium.

Secondly, there is the existence of the complex internal circulation of the gland with a portal system carrying blood rich in steroid hormones to the medulla.

Thirdly, the work of Shepherd and West (1951) and Coupland (1953) drew attention to the correlation between the cortex to medullary size ratio and the percentage of adrenaline in the glands of various mammalian species. More recently, Pohorecky and Wurtman (1971) have shown in the rat that the high concentration of glucocorticoids delivered to the medulla from the cortex is essential for the maintenance of the enzyme, Phenylethanolamine-N-methyl transferase (PNMT) which methylates noradrenaline to adrenaline.

Finally, there is a little evidence from studies on adrenal gland catecholamine content that corticotrophin administration results in the depletion of adrenaline but not noradrenaline. ( Houssay et al (1933) and Eranko (1952) ).

The blood supply to the medulla is from two main sources. There are the capillary channels of the cortical zona reticularis which become the sinusoids of the medulla. Also the arteriae medullae pass through the cortex from the "capsular" plexus before breaking up into capillary channels which form a network around adjacent chromaffin cells. Capillary blood vessels derived primarily from the arteriae medullae anastomose with each other and the branches of the venous tree ( Flint, 1900 ). Thus not all the blood flowing through the arteriae medullae enters the cortico-medullary sinuses as it can also pass directly to the central vein through the venae medullae.

The anatomy of the musculature of the adrenal blood vessels is complex and not fully understood but one wonders if it could alter the distribution of flow between the cortico-medullary sinusoids and the arteriae medullae. This would change the concentration of glucocorticoids bathing the medullary cells.

When Shepherd and West (1951) surveyed the percentage of adrenaline in the adrenal medulla of various mammalian and avian species they found a good correlation with the ratio of the volume of cortical to medullary tissue. In species where this ratio is high, such as in the cavy and rabbit, the medulla contains almost exclusively adrenaline while in those with a lower ratio, such as the cat and dog, a greater percentage of noradrenaline is found. Moreover, the medulla of foetal mammals contains very little adrenaline and the adrenal content of this catecholamine rises sharply after birth in parallel with a period of marked cortical development. The dependence of methylation on the cortex only appears to be the case in mammals. Other vertebrates which have separate steroid hormone and catecholamine synthesising tissues, have chromaffin glands containing a large proportion of adrenaline (Shepherd, West and Erspaner, 1953)

Pohorecky and Wurtman (1971) have established that in the mammalian adrenal medulla, the maintenance of the PNMT enzyme depends on the delivery of a high concentration of glucocorticoids via the corticomedullary portal system. Their evidence is summarised in two reviews (1971, 1972) and I shall now mention the salient features.

Most of the studies were done in rats.

Hypophysectomy results in a rapid fall in medullary PNMT activity with a half life of three to five days.

This activity is restored by corticotrophin administration but not glucocorticoid maintenance therapy.

Enzyme activity only begins to be restored by the administration of amounts of glucocorticoids which give peripheral arterial concentrations fifty to one hundred times normal.

Thus the high concentrations of glucocorticoids found in the adrenal medulla ( one hundred to two hundred times peripheral concentrations ) appear to be necessary for the maintenance of PNMT and the mechanism is thought to be related to the synthesis of the enzyme protein.

Other classes of steroid hormones do not appear to have a physiological action on PNMT activity.

However, it is noteworthy that they base little of their argument on the actual catecholamine content of these glands. This is, no doubt, because they found that changes in catecholamine content lag behind the changes in enzyme activity. After hypophysectomy, adrenaline content does decline but with <sup>noradrenaline</sup> a half life of thirty to eighty days while/does show an increase but not in proportion to the fall in adrenaline. However, radioactive tracer studies have shown that the half life of adrenaline in intact rat adrenal medulla is seven to fourteen days ( Udenfriend and Wyngaarden, 1956 ).

The discrepancy between the half life of adrenaline in intact and hypophysectomised animals can be explained by postulating that adrenaline synthesis still



continues at a near normal rate in spite of the very low PNMT levels found after one to two weeks. However, a much simpler explanation is that the release of adrenaline is considerably reduced.

We decided to investigate whether there is such a humoral component to adrenal catecholamine release. Our studies were all done in dogs, usually greyhounds or crossbred collies.

We found that intravenous Synacthen (corticotrophin-like peptide) resulted in a prolonged release of adrenal catecholamines from both intact and denervated glands.

Chemoreceptor stimulation has been shown to cause reflex release of corticotrophin from the anterior pituitary gland resulting in a high output of corticosteroids from the adrenal cortex ( Anichkov et al. (1960) and Marotta (1972) ). We wondered whether the pituitary-adrenocortical axis could be involved in the response of the adrenal medulla to chemoreceptor stimulation. Using prolonged chemoreceptor stimulation lasting ten to twenty minutes, we found that as well as the expected immediate neuronally mediated catecholamine release, there was also a delayed component which was still present after denervation of the gland. This release occurred seven to ten minutes after commencement of chemoreceptor stimulation and had a magnitude and prolonged time course similar to that seen with Synacthen administration.

The delayed release of catecholamines in response to both Synacthen and chemoreceptor stimulation was inhibited by cycloheximide which has been shown to block steroidhormone secretion ( Garren, Ney and Davies, 1965 ).

However, cycloheximide had no effect on the immediate neuronally mediated medullary release in response to chemoreceptor or baroreceptor reflex stimulation. Furthermore the drug had no effect on the catecholamine output from isolated perfused adrenal glands.

Finally we demonstrated that hydrocortisone in the concentrations expected in the adrenal gland during stress increased the catecholamine output from isolated Locke solution perfused adrenal glands in a dose dependent manner.

Thus we concluded that a humoral mechanism involving the anterior pituitary - adrenal cortical axis can release adrenal medullary catecholamines ( Critchley and Ungar(1974), Critchley et al(1975,1981) ).

In our anaesthetised animals we found that a  $P_{O_2}$  of twenty to thirty torr was required to activate the neuronal reflex while the humoral mechanism was elicited by less severe but prolonged hypoxia in the region of fifty torr. Anaesthesia is known to depress reflexes and thus in the non-anaesthetised animal we would expect to find that the reflexes are activated by milder hypoxia. The lower threshold for the humoral mechanism and evidence in the literature which I have already discussed, suggests that the adrenal cortex exerts a tonic influence on medullary catecholamine output. This might even explain, by a process of feedback induction, the dependence of the methylating enzyme on the integrity of the anterior pituitary - adrenal cortical axis.

In conclusion, I propose that the body has at least three mechanisms through which the adrenal glands respond to a stress such as hypoxia.

Firstly, there is the humoral pathway involving the anterior pituitary - adrenocortical axis. This comes into play with mild prolonged hypoxia.

With more severe hypoxia there is the neuronal reflex which can respond rapidly in the emergency situation.

Finally there is the possibility of a direct effect of hypoxia acting locally on the adrenal medulla. This last mechanism is probably of little physiological significance except under extreme circumstances such as asphyxia.

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