J. Physiol. (1963), **168**, *pp.* 939–944
With 1 *text-figure*
Printed in Great Britain

CATECHOLAMINE-CONTAINING STRUCTURES IN THE HYPOGASTRIC NERVES OF THE DOG

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(*Received* 22 *February* 1963)

The present investigation arose from the search for a post-synaptic adrenergic nerve of sufficient size to allow accurate comparison of the time course of the depleting action of reserpine on catecholamines in adrenergic axons and their cell bodies. The hypogastric nerves of the dog were chosen on account of their size, but their variable and high content of adrenaline (Muscholl & Vogt, 1958) suggested that the anatomical structure of the 'nerve' was complex. This was supported by preliminary observations made on six young dogs, five of which retained a high catecholamine content of the hypogastric nerves after a large dose of reserpine.

The purpose of the subsequent experiments was to identify and locate the structures containing the large amounts of reserpine-resistant catecholamines and to see whether a part of the nerve could be found which consisted of nothing but fibres. The methods employed were chemical analysis of the catecholamines in different parts of the nerve and histological examination after staining for chromaffin tissue.

METHODS

Dogs of both sexes and various ages were bled under ether or pentobarbitone. The inferior mesenteric ganglia and the hypogastric nerves were dissected out, often including some swellings situated where the nerves enter the pelvic plexus.

Catecholamine estimations. The hypogastric nerves were severed from the ganglia, subdivided in different ways (see below), and the ganglia and the portions of nerve weighed, homogenized and analysed for catecholamines. When it was not possible to extract the tissue immediately, it was kept intact at $-17°$ C for up to 3 days. The methods of extraction of the amines, their paper-chromatographic separation and estimation by bioassay were essentially the same as those used by Vogt (1954), but an attempt was made to use the new adrenolytic drug Nethalide (I.C.I.; 2-isopropylamino-1-[2-naphthyl]ethanol hydrochloride; Black & Stephenson, 1962) in order to improve the bioassays of adrenaline. Since this compound has been reported to be nearly free from sympathomimetic activity, it should be useful as a specific inhibitor of the response of the rat's uterus to adrenaline, thus making that bioassay more specific. However, in concentrations in the bath of 10^{-8} or lower it did not consistently antagonize the inhibition by adrenaline of the contractions elicited by carbachol; in

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concentrations of 10^{-7} and 10^{-6} the drug exerted an inhibitory effect on the carbachol contractions themselves. It therefore did not seem practicable to employ Nethalide as a specific antagonist in this assay.

However, Nethalide proved useful in sensitizing the pithed rat to the pressor effect of adrenaline. An intravenous dose of 1 mg/kg, while not altering the response to noradrenaline, changed the biphasic and poorly graded responses to adrenaline into larger, purely pressor, responses; in this way, a good dose–response curve and better discrimination were obtained at very small doses of adrenaline, down to 1 and 0·5 ng. An example is given in Fig. 1.

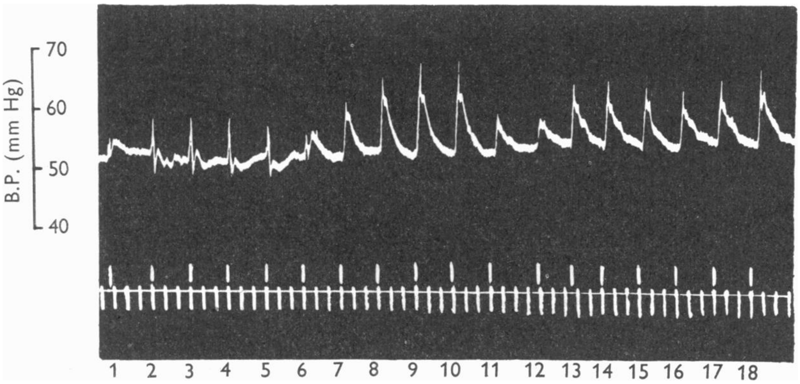

Fig. 1. Assay of adrenaline on the carotid blood pressure of a pithed rat (170 g body wt.) after an injection of Nethalide. Time marker, minutes. At each upward stroke an intravenous injection of 0·3 ml. total volume was given. The solutions were injected in the following order: (1) 0·9 % NaCl; (2–5) 3, 5, 7, 9 ng adrenaline; (6) Nethalide HCl 1 mg/kg; (7–12) 3, 5, 7, 8, 1, 0·5 ng adrenaline; (13) 0·05 ml. nerve extract (adrenaline region of chromatogram); (14) adrenaline 3 ng; (15) 0·05 ml. extract; (16) adrenaline 2 ng; (17) 0·05 ml. extract; (18) adrenaline 4 ng.

The method of estimation was checked by recovery experiments. In some of these known amounts of adrenaline or noradrenaline were added to one half of a homogenate from the inferior mesenteric ganglion and the difference in catecholamine content between the two portions was measured. In the others, known quantities of amines were added to homogenates of rat cerebral or cerebellar cortex, tissues which contain only negligible amounts of these amines. The recoveries ranged usually between 60 and 70 %.

Histology. Longitudinal sections were prepared of the inferior mesenteric ganglia and the hypogastric nerves of four dogs, two adults and two puppies of either sex. The tissue was stretched out and tied to a glass plate and fixed for 24 hr in the dark in a mixture of 10 ml. 40 % formaldehyde and 90 ml. of a solution of 2·5 g potassium bichromate and 1 g sodium sulphate in 100 ml. water. After washing the tissue overnight it was embedded in paraffin, sectioned at 6 μ and stained with cresyl violet. The chromaffin cells are stained greenish-yellow after this treatment.

RESULTS

The catecholamine content of the hypogastric nerves

Table 1, experiments 1–6, shows the catecholamine concentration found in the hypogastric nerves of the six young dogs referred to above. The animals, aged 2 and 4 months, were injected with reserpine 0·7 mg/kg and examined after an interva lranging from 4 to 19 hr. In spite of the treatment with reserpine, the amine content of the nerves of puppies 1–5 was higher than that of a group of untreated adult dogs (Nos. 7–11, Table 1); only in puppy No. 6 was there the low concentration of noradrenaline to be expected in an adrenergic nerve after an injection of reserpine.

The subsequent experiments were designed to test whether the use of young dogs was responsible for the abundance of reserpine-resistant catecholamines, or whether the nerves had been dissected out in such a way as to include groups of cells from the inferior mesenteric ganglion. This ganglion was shown by Kohn (1903) to contain much chromaffin tissue, some of which might have 'contaminated' the upper part of the hypogastric nerves whereas the lower part might be free from such tissue. In fifteen adult dogs, therefore, both hypogastric nerves were severed from the mesenteric ganglion just below its lower pole and divided into proximal and distal parts of approximately equal length. The results of the estimations of noradrenaline and adrenaline in the two parts of the nerves are shown in Table 2, rows 1 and 3. It is useful to compare these figures with those of row 5, which contains the analyses of the inferior mesenteric ganglia of seven adult dogs; the range is greater than that of a series reported earlier by Muscholl & Vogt (1958), one ganglion containing as much as 131 μg noradrenaline and 82 μg adrenaline/g tissue; as discussed by these authors, those variable concentrations reflect the irregular occurrence of chromaffin bodies in the ganglion. The most striking fact about the catecholamine content of the two portions of the hypogastric nerves of the adult dogs is the extremely wide range of the figures which, for adrenaline, overlap with those of the

TABLE 1. Catecholamine content (μg/g fresh tissue) of hypogastric nerves of dogs

No. of dog	Treatment	Age (months)	Noradrenaline	Adrenaline
1–5	Reserpine 0·7 mg/kg I.V.	2–4	4·8–33·0	0·6–3·3
6	Reserpine 0·7 mg/kg I.V.	2–4	0·6	0·09
7–11	None	Adult	1·3–3·6	0·09–1·5

TABLE 2. Catecholamine concentrations (μg/g fresh tissue) in the hypogastric nerves and inferior mesenteric ganglion of the dog

Tissue		Noradrenaline: No. of dogs	Noradrenaline: Mean and range	Adrenaline: No. of dogs	Adrenaline: Mean and range
Hypogastric nerves, proximal half	Adult	15	3·51 (0·14–9·52)	14*	< 1·33 (< 0·03–9·52)
	Puppies	2	0·6; 1·0	2	< 0·5; < 0·6
Hypogastric nerves, distal half	Adult	15	4·14 (0·20–16·20)	14*	2·10 (0·05–13·50)
	Puppies	2	1·0; 1·0	2	< 0·5; < 0·5
Ingerior mesenteric ganglia	Adult	7	76·6 (25·4–131·0)	7	31·0 (3·7–81·9)

* The adrenaline fractions of one dog were lost.

ganglion. The results indicate that the high amine concentrations recorded for whole nerves in Table 1 are not explained by the use of puppies, but may occur at random among adult dogs as well. In the experiments recorded in Table 2 the concentrations found in the nerves of two puppies (rows 2 and 4) were in the lower range of the figures for adults.

Proximal and distal halves of the hypogastric nerves did not differ consistently in their catecholamine content; not only were means and ranges rather similar (rows 1 and 3, Table 2), but for eight instances in which the proximal half contained more catecholamines than the distal half there were seven in which it contained less. In one additional experiment the nerves were divided into three equal parts, as it was conceivable that the high amine content in the distal half might be contributed by the beginning of the pelvic plexus, known to contain ganglia and chromaffin bodies. However, the part of the nerves which included the beginning of the pelvic plexus contained just a little less, and not more, catecholamines than the middle part of the nerves.

It follows from these results that it was not possible to find a region of the hypogastric nerves which had a consistently low catecholamine content and was therefore presumably free of chromaffin tissue and suitable for the analysis of drug action on the catecholamines in axons. The problem remained of the actual site of the structures which were responsible for the chemical findings.

Histological structure of the hypogastric nerves

Langley & Anderson (1894) have shown that the hypogastric nerves of the cat and the rabbit consist of about 90% non-myelinated and 10% (afferent) myelinated fibres. As these authors also pointed out (1895), a proportion of the non-myelinated fibres are preganglionic and are interrupted in ganglia situated usually near the innervated organ, such ganglia being more frequent in the rabbit than in the cat. There is no mention of the occurrence of any cells in the nerve proper. The presence of chromaffin tissue in sympathetic ganglia, particularly in those of the abdomen, and in the pre-aortic nerve plexus of cat and man, was known since the work of Kohn (1903). However, whereas in man there is only a hypogastric plexus and no hypogastric nerve, the dog has distinct hypogastric nerves, which are 4–8 cm long and have few branches and no macroscopic swellings. The question, therefore, arose, where chromaffin tissue, if such was present, might be located.

Longitudinal serial sections were made of the inferior mesenteric ganglia and the hypogastric nerves of four dogs, two adults and two puppies of either sex. As expected, large chromaffin bodies were found inside the inferior mesenteric ganglia, but these did not extend into the hypogastric nerve. However, long, slim clusters of chromaffin cells were occasionally seen to lie on the surface of the hypogastric nerve underneath the epineurium. Furthermore, in three dogs small elongated groups of about 5–50 chromaffin cells occurred within the very core of the nerve; there were many blood vessels in the immediate vicinity of these groups. They were usually found in the middle part of the nerve, 2 cm or more away from either the lower pole of the mesenteric ganglion or the beginning of the pelvic plexus (see Vogt, 1963, Fig. 1). They were not necessarily near any nerve cells. Kohn (1903) has described chromaffin cells located in the same way inside bundles of fibres of the pre-aortic plexus.

Nerve cells were abundant in the meshes of the pelvic plexus, and were found in large clumps at the origin of the hypogastric nerves near the lower pole of the inferior mesenteric ganglion. They were also present, though scarce, within the hypogastric nerve proper, and were usually arranged like a string of beads in the core of the nerve.

As far as can be judged from this small material there was no difference in the amount of chromaffin tissue in the nerves of young and of adult dogs. Similarly, Kohn (1903) found that chromaffin tissue does not degenerate in adult cats and rabbits.

DISCUSSION

The concentrations of catecholamines in the hypogastric nerves are expressed per gram of fresh tissue. This introduces a small error due to the difficulty of dissecting all the fat and blood vessels off the nerves, thus making some of the concentrations appear too low. Using the catecholamine concentrations in other parts of the sympathetic system of the dog as a guide (Vogt, 1954), one would expect post-ganglionic adrenergic fibres to contain noradrenaline in a concentration not greatly exceeding 2·4 μg/g. Some of the lower figures obtained in the present work may indicate that these nerves contained non-adrenergic afferent and preganglionic fibres. The mean noradrenaline content of the superior cervical and stellate ganglia was found earlier to be, respectively, 6·8 and 4·7 μg/g. Thus, the presence of nerve cells found to lie between the fibres of the hypogastric nerve might produce some increase in the concentration of noradrenaline above that of adrenergic fibres.

In one third of all the estimations the adrenaline content lay between 0·9 and 13·5 μg/g, and thus indicated the presence of many chromaffin cells. These cells were arranged in long, narrow groups either within or on the surface of the nerve, so that there was no macroscopic indication of their presence. And since these cells are likely to contain a certain percentage of noradrenaline, it is not surprising that the two portions of hypogastric nerve with the highest content of adrenaline had also the highest content of noradrenaline, 9·5 and 16·2 μg/g. The occurrence in the hypogastric nerve of the dog of nerve cells and chromaffin cells which vary greatly both in location and in number must be taken into account in any pharmacological experiments on this tissue.

SUMMARY

1. The noradrenaline concentration in the proximal half of the hypogastric nerves of a series of fifteen adult dogs ranged from 0·14 to 9·5 μg/g fresh tissue, that of the distal halves from 0·2 to 16·2 μg/g; the adrenaline concentrations were even more variable.

2. An adrenaline concentration above 0·15 μg/g occurred five times in the proximal and six times in the distal part of the nerve. This pointed to chromaffin tissue occurring at random in either part of the nerve.

3. Histological examination showed chromaffin cells forming elongated clusters either on the surface of the nerve under the epineurium, or in the core of the nerve, more often in its middle part than in the vicinity of the inferior mesenteric ganglion or of the pelvic plexus. This chromaffin tissue accounts for the frequent occurrence of reserpine-resistant catecholamines in the hypogastric nerves of the dog. Its occurrence is not restricted to puppies.

4. Nerve cells, usually aligned in single file, were found in any part of the hypogastric nerve; only sometimes did they lie next to the chromaffin cells.

5. Within the limited material examined puppies did not show larger catecholamine concentrations and greater amounts of chromaffin tissue than adult dogs.

We are greatly indebted to Mr B. S. C. Hollands for his skilful preparation of the sections. We wish to thank Dr A. Spinks for a gift of Nethalide. The research reported in this paper has been sponsored in part by the Air Force Office of Scientific Research, O.A.R., through the European Office, Aerospace Research, United States Air Force. The work was done during tenure by one of us (S.V.) of a British Council Scholarship.

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