Regional variations in the distribution of noradrenaline along the rat vas deferens

The vas deferens is widely used as a model to study different aspects of adrenergic mechanisms due to the rich sympathetic innervation of its smooth muscle layers (Falck, 1962). The density of this adrenergic supply varies morphologically between the prostatic and the epididymal ends in several species. Sections of vas deferens processed histochemically for the demonstration of monoamines show the population of adrenergic nerves to be denser in the prostatic than in the epididymal end (Norberg, 1967; Owman & Sjöstrand, 1965; Sjöstrand, 1965; Norberg, Risley & Ungerstedt, 1967; Bell & McLean, 1970). These observations agree with ultrastructural studies which demonstrate variations in the amount of nerve fibres present in both ends of the vas deferens (Farrell, 1968). For example, in equivalent surfaces of thin sections through the muscle layers, 152 endings were identified in the prostatic end, 121 in the medial third and 52 in the epididymal end.

In the course of experiments in which slices of vas deferens of the rat were incubated *in vitro* (Zieher & Jaim-Etcheverry, unpublished), we noticed a great diversity in the noradrenaline content of slices in different incubation flasks. We now report the distribution of noradrenaline in thin transverse segments obtained from the entire length of the vas deferens.

Vasa deferentia were removed from Wistar rats of 250–300 g along with a portion of the prostate and the epididymis. For each experiment, groups of 4–5 vasa deferentia were pinned together at their *in situ* length (38–40 mm) on dental wax and sectioned with a special chopper consisting of 20 stainless steel razor blades whose cutting edges were maintained exactly 2 mm apart by interposing between them acrilic plates of the appropriate thickness. The entire vas deferens was sectioned in one operation so that 19–20 tissue segments of 2 mm thickness were obtained from the complete length of the organ. The segments corresponding to the various vasa deferentia cut by two

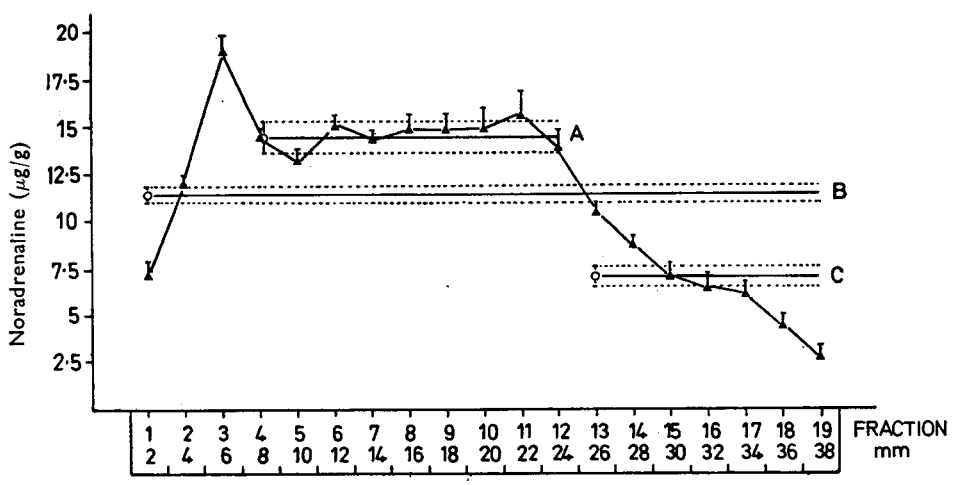

FIG. 1. Concentration of noradrenaline assayed in 2 mm segments obtained from the entire length of the vas deferens of the rat. Fractions are numbered starting from that corresponding to the prostatic end of the organ. Triangles represent mean values ±s.e. from results of 5 experiments. Mean values ±s.e. (dotted lines) corresponding to segments comprised between fractions 4–12 are represented in A, to segments 1–19 (entire length of the organ) in B and to segment between fractions 13–19 in C. Significance of differences between A and B, $P < 0{\cdot}05$; between B–C and A–C, $P < 0{\cdot}001$.

adjacent blades and found between them were pooled together, weighed and homogenized in 0·4 N perchloric acid for extraction of noradrenaline. Fractions were numbered 1 to 19 starting from that of the prostatic end. Tissue extracts were purified by column chromatography on Dowex 50W-X4 resin (column size 4·2 × 50 mm), eluted with HCl and measured fluorometrically (Häggendal, 1963). The fluorescence was read at 400–515 nm (excitation and emission wavelengths respectively) and the recovery of noradrenaline was 90%.

Three different zones may be identified along the vas deferens (Fig. 1). The prostatic end of the organ (except the 2 mm corresponding to its termination in the prostate) comprising the following 6–8 mm, has a high concentration of noradrenaline (18·9 μg/g in fraction 3). From there-on the concentration of noradrenaline remains stable at a constant level (between 13·2 and 15·7 μg/g) for the following 18 mm, and in fraction 12 initiates a progressive and sustained decline, reaching the lowest level at the epididymal end (6·5 μg/g in fraction 19). The mean value obtained for grouped data corresponding to all fractions is 11·5 $\pm$ 0·4 μg/g, while the mean value of the segment comprising fractions 4–12 is 14·5 $\pm$ 0·8 μg/g and that of fractions 13–19 is 7·0 $\pm$ 0·9 μg/g. The differences between the values of both segments are statistically significant and they also differ significantly from the mean value for the entire organ.

The marked differences observed in the regional distribution of noradrenaline in rat vas deferens confirm previous morphological experiments since the quantity of noradrenaline correlates with the density of adrenergic innervation in sympathetically innervated tissues. Moreover, they reflect the lack of homogeneity of the nervous supply within a given tissue. The preganglionic fibres of the hypogastric nerve penetrate the vas deferens for the initial 6–8 mm where postganglionic neurons lie in close vicinity to the muscle coat of the organ (Sjöstrand, 1962, 1965; Ohlin & Strömblad, 1963; Owman & Sjöstrand, 1965). The presence of this nervous plexus is probably responsible for the peak in noradrenaline concentration found at this level. It is in this zone where the adrenergic nerve fibres are first apparent during development of the innervation and from where they extend to the rest of the organ (Furness, McLean & Burnstock, 1970). This possibility of distinguishing biochemically between zones of the organ where the adrenergic cell bodies or their terminals are localized, might prove to be useful in the analysis of the effects of drugs which have differing effects on the concentration of noradrenaline in both portions of the adrenergic neuron.

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An interaction between hydrocortisone and hemicholiniums in mice

Toxicity of hemicholinium-3 (HC-3) has been attributed to failure of acetylcholine synthesis due to interference with the passage of choline to its intracellular sites of acetylation (MacIntosh, Birks & Sastry, 1958; Gardiner, 1961). The work of Schueler (1955) and Reitzel & Long (1959), who have reported that choline is the specific antagonist to HC-3 toxicity, supports this. Perfusion studies on the cat superior cervical ganglion have shown that the presence of choline in the perfusion fluid is essential for optimal synthesis and release of acetylcholine and that acetylcholine synthesis is inhibited by HC-3 (Birks & MacIntosh, 1961). Drugs which can influence the plasma levels of choline might therefore be expected to modify the toxicity of HC-3. Cortisone has been reported to lower the plasma choline of dogs by 60–80% within 30 min of injection (MacIntosh, 1963). We now report the effects of a water-soluble hydrocortisone derivative (hydrocortisone sodium succinate) on the toxicity in mice of HC-3 and its *p*-terphenyl analogue (TPHC-3) (Gardiner & Lee, 1969).

Albino mice of either sex weighing 16–24 g were used. The mice were pretreated with hydrocortisone (10 mg/kg) or 0·9% sodium chloride (saline). One h later the mice were injected with different doses of HC-3 or TPHC-3. 20 mice were used for each dose.

All drugs were made up in saline and administered by intraperitoneal injection. The volume of drug solutions injected was 0·1 ml per 10 g mouse. The number of mice that died at the end of 2 h were noted.

The mortality in mice increased with increasing doses of HC-3 and TPHC-3 (Table 1). TPHC-3 was the more toxic (Gardiner & Lee, 1969). Pretreatment with hydrocortisone (10 mg/kg) for 1 h reduced the mortality produced by all doses of HC-3 and TPHC-3.

It was anticipated that, if hydrocortisone produced a fall in plasma choline, as

Table 1. *Partial protection of mice against HC-3 and TPHC-3 by pretreatment with hydrocortisone* (10 mg/kg). Hydrocortisone or saline was administered 1 h before HC-3 or TPHC-3. The values given are % mice dead 2 h after injecting HC-3 or TPHC-3 (20 mice/group) and each value represents the mean ± s.e. of four experiments.

HC-3			TPHC-3		
Dose (μg/kg)	Animals receiving: hydrocortisone	saline	Dose (μg/kg)	Animals receiving: hydrocortisone	saline
120	16 ± 2	29 ± 4	80	10 ± 5	19 ± 6
150	33 ± 3	53 ± 9	100	16 ± 5	45 ± 8
180	55 ± 3	70 ± 5	120	63 ± 3	76 ± 4
220	81 ± 3	90 ± 0			