The effect of 6-hydroxydopamine on contractions produced by sympathomimetic amines in the human isolated vas deferens

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The effects of a single intravenous dose of 6-hydroxydopamine on the peripheral adrenergic nerve plexus in the mouse and rat vas deferens have been described by Furness et al (1970). After 100 mg kg^{-1} there were spontaneous contractions during the first hour, the fluorescence intensity was reduced strongly in all nerves after 4–6 h, while after 24 h only 10–40% of the adrenergic nerves remained. Transmission failed 2–2·5 h after the injection of 250 mg kg^{-1}. Nerves in the vas deferens appear to be more resistant to the effects of 6-hydroxydopamine than do nerves in the atria, iris or spleen (Thoenen & Tranzer 1968; Malmfors & Sachs 1968), perhaps because of a poorer blood supply (Sachs 1971). For this reason, 6-hydroxydopamine has been administered in vitro, where it produces destruction of the adrenergic nerves in the vas deferens (Jonsson & Sachs 1970) and failure of transmission (Furness 1971; Wadsworth 1973). In these in vitro experiments, ascorbic acid was used to delay oxidation of 6-hydroxydopamine to the quinone (Heikkila & Cohen 1975) although in fact this may not be necessary and in addition ascorbic acid may produce pharmacological actions of its own (Gillespie & McGrath 1975). We have examined various treatment schedules to find a method of selectively destroying adrenergic nerves in the human isolated vas deferens, avoiding the use of ascorbic acid.

Specimens of human vasa deferentia (10–30 mm long) were obtained from patients undergoing elective vasectomy under local anaesthesia. The operations were done between 16.00 and 18.00 h. The tissues removed were immediately placed in iced Krebs-Henseleit solution and stored at 4 °C until use the following morning.

The vasa were suspended in 7·5 ml baths under a resting tension of 1·0 g. Contractions were isometrically recorded with Grass FTO3C or Washington UF1 gauges using a Grass 7B or Washington 400 MD2 recorder. The physiological salt solution used for transportation, storage and during the experimental investigation had the following composition (mM); Na^+ 143, K^+ 5·8, Ca^{2+} 2·6, Mg^{2+} 1·2, Cl^- 128, $H_2PO_4^-$ 1·2, HCO_3^- 25, SO_4^{2-} 1·2, and glucose 11·1. It was gassed with 5% CO_2 in oxygen and maintained as 36–38 °C throughout the experiment. An equilibrium period of 3–5 h was allowed between setting up the vasa and starting the experiment.

Control responses were obtained using KCl or methoxamine. In one preparation, the vas deferens was electrically stimulated through platinum ring electrodes using a Grass S4K stimulator for 5 s at a frequency of 10 Hz with impulses of 0·5 ms duration and 70V. Tyramine was then added in a concentration producing a maximal response (which consisted of rhythmic contractions). This was followed by 6-hydroxydopamine HBr in a final concentration of 1000 $\mu g\ ml^{-1}$. After 15–20 min, the preparation was washed and the administration of 6-hydroxydopamine and tyramine repeated in the same concentrations as used previously. Washout followed by replenishment of the 6-hydroxydopamine (and tyramine) was repeated every 15–20 min up to a maximum of 8 such treatments until the tyramine effect was completely abolished. Similar experiments were performed using norephedrine in place of tyramine. When the rhythmic contractions had disappeared, KCl or methoxamine was added to assess whether the treatment had caused any damage to the muscle.

A separate series of experiments was done to eliminate the possibility that the rhythmic contractile activity diminished spontaneously over the time course of the 6-hydroxydopamine treatment. Rhythmic contractile activity was initiated by tyramine in 8 vasa and by norephedrine in 3 more vasa. Provided either drug remained in contact with the tissue, contractile activity continued with no sign of fatigue for the entire duration of the experiment (4–6 h).

The drugs used were methoxamine hydrochloride (Burroughs Wellcome), tyramine hydrochloride, (—)-noradrenaline hydrochloride and 6-hydroxydopamine HBr (Sigma).

The concentrations quoted in the text are the final bath concentrations expressed as $\mu g\ ml^{-1}$ of the salt. 6-hydroxydopamine HBr was dissolved in 0·9% NaCl containing 0·1 M HCl immediately before addition to the bath.

Addition of KCl before 6-hydroxydopamine produced a sustained contraction (0·25–2·5 g) which was maintained until wash-out. In a few preparations, repeated phasic contractions were superimposed on it. Although the response to potassium was reduced after 6-hydroxydopamine in some experiments, in others it was increased, and the mean effect showed little change (Table 1).

After treatment with 6-hydroxydopamine, most preparations were tested with methoxamine, which elicited rythmic contractions similar in size to those produced in untreated vasa (see methods). In two preparations methoxamine was added before and

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Table 1. Effect of 6-hydroxydopamine on the contractility of the isolated human vasa deferentia. The first part shows the control responses obtained with 150 mM potassium, 5–50 $\mu g\ ml^{-1}$ methoxamine or by electrical stimulation of the postganglionic neurons via ring electrodes. In the second part is indicated the number of doses (100 $\mu g\ ml^{-1}$) of 6-hydroxydopamine (repeated at 15–20 min intervals) required to abolish contractions induced either by norephedrine or by tyramine. The third part shows the magnitude of responses obtained with the original doses of potassium or methoxamine following 6-hydroxydopamine treatment.

Before 6-Hydroxydopamine					6-Hydroxydopamine treatment		After 6-Hydroxydopamine			
		Methoxamine response						Methoxamine response		
Length of vas (mm)	Potassium response amplitude (g)	Amplitude (g)	Frequency contraction min^{-1}	Electrical amplitude (g)	Indirectly acting drug used	No. of 6-hydroxydopamine treatments	Potassium response amplitude (g)	Amplitude (g)	Frequency contraction min^{-1}	Dose of methoxamine $\mu g\ ml^{-1}$
20	0·25				Norephedrine	8		0·25	0·528	10
22	0·75	5	0·48		Norephedrine	8		4·0	1·66	10
18	1·0				Norephedrine	7		4·2	1·5	10
10	0·25				Norephedrine	7		0·5	0·736	10
16	1·0				Tyramine	5	2·0			
11	0·40				Tyramine	6	2·0			
20	1·0			6·25	Tyramine	7		4·0	0·369	5
15	0·8				Tyramine	7	0·75	0·75	0·21	5
30	2·5				Tyramine	8*	1·0			
15		1·0	0·24		Tyramine	6	0·5	1·5	0·20	50
30	1·5				Tyramine	7	0·75	1·0	0·40	50

* 90% abolished

after 6-hydroxydopamine, and in both cases the responses were similar to one another (Table 1).

Tyramine or norephedrine cause repeated rhythmic contractions that continued for at least 4–6 h with no sign of fatigue (Ratnasooriya et al 1979). These contractions, as well as the response to electrical stimulation, were completely abolished in 10 out of 11 vasa following 6-hydroxydopamine treatment (Fig. 1). In the remaining experiment treatment was stopped after 8 applications of 6-hydroxydopamine, at which time the response to tyramine was 90% abolished. An augmentation of tyramine or norephedrine induced contractions was usually seen during the first 1–3 treatments with 6-hydroxydopamine. The time required for the complete abolition of contractions was 1·25–2 h.

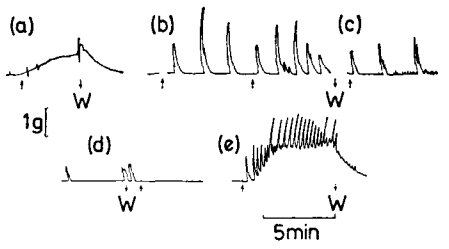

FIG. 1. Human isolated vas deferens. (a) A control response was obtained by addition of KCl (150 mM ↑). (b) The unstimulated vas was quiescent, but rhythmic contractions were induced by tyramine HCl (50 $\mu g\ ml^{-1}$ ↑). The contractions shown are for the final part of the 30 min contact period allowed initially for stabilization. 6-Hydroxydopamine HBr (1000 $\mu g ml^{-1}$ ↑) was then added. After the 3rd readministration of 6-hydroxydopamine and tyramine (↑, c), the contractions were reduced in size. (d) This panel shows the last part of the 5th exposure to 6-hydroxydopamine and tyramine and the 6th readministration (↑) which abolished contractions. (e) A final response to KCl (↑) showed that the muscle was still responsive. ↓ W = washout.

The motor innervation of the human vas deferens is believed to be adrenergic, since isolated preparations can be contracted by tyramine (Birmingham 1968) and the response to field stimulation is blocked by α-adrenoreceptor antagonists (Anton & McGrath 1977). In various tissues 6-hydroxydopamine has been shown to destroy the adrenergic ground plexus, and this is accompanied by the loss of fluorescence, loss of responsiveness to indirectly acting sympathomimetic amines, loss of noradrenaline accumulating ability and transmission failure (for review see Kostrzewa & Jacobowitz 1974). In our experiments, we used abolition of the response to tyramine as an indicator and found that this could be produced by repeated application of 6-hydroxydopamine and was accompanied by loss of the response to nerve stimulation. Abolition of the tyramine response cannot be explained by tachyphylaxis as we have shown that tyramine-induced rhythmic contractions will continue without fatigue for at least 4–6 h.

We thought it of value to use a treatment schedule omitting ascorbic acid, but this necessitated increasing the concentration of 6-hydroxydopamine in order to obtain complete abolition of the tyramine response. Presumably the effective concentration of 6-hydroxydopamine was considerably less, due to oxidation, than the initial bath concentration. Considering the high concentration used, it might be expected that there would be general depression of the muscle or α-adrenoceptor antagonism (Haeusler 1971). However, the treatment satisfied our criteria for being specific since responses to methoxamine and to KCl were not reduced. Westfall & Fedan (1975), in fact, reported potentation of methoxamine, but this was due to development of post-junctional supersensitivity that took several days to appear.

Although 6-hydroxydopamine was replenished at 15 min intervals, the total time required for in vitro denervation was not much different from that reported

by Jonsson & Sachs (1970), Furness (1971), Wadsworth (1973), Gillespie & McGrath (1975) or Aprigliano & Hermsmeyer (1976). Thus there may be a minimum time of 1–2 h required for destruction of adrenergic nerves with 6-hydroxydopamine.

Norephedrine is a sympathomimetic drug (Goodman & Gilman 1970). Since the response to norephedrine was abolished by 6-hydroxydopamine, we conclude that it is predominantly an indirectly-acting sympathomimetic amine, according to the classification of Fleckenstein & Burn (1953).

It is concluded that this treatment schedule is an effective method for selective destruction of adrenergic nerves in the human vas deferens in vitro. It is particularly suitable for human tissues where in vivo treatment would be impossible.

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Genetic evidence for the involvement of different oxidative mechanisms in drug oxidation

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In general, the metabolic oxidation of drugs is considered to be mediated by the cytochrome P450 monooxygenase system found in the liver and other tissues. However, it is by no means clear whether or not the oxidation of a given substrate in man is achieved by one or more similar forms of this enzyme, or indeed by a different enzyme not involving cytochrome P450. Recently, metabolic pharmacogenetic studies have demonstrated that the carbon oxidation of three structurally dissimilar drugs is under the control of a single gene locus and exhibits polymorphism (Sloan et al 1978b). It was shown in these investigations that a clearly identifiable proportion of the population (some 5% of British whites), who were phenotypically poor hydroxylators of debrisoquine (Mahgoub et al 1977), were also defective hydroxylators of guanoxan and deficient *O*-de-ethylators of phenacetin.

In this communication, we describe the conversion of acetanilide to paracetamol by aromatic hydroxylation in volunteers who gave their informed consent and who had been previously phenotyped with respect to their ability to oxidize oral doses of debrisoquine, guanoxan and phenacetin.

The eight healthy adult males (age 22–42) who had previously been phenotyped with debrisoquine, comprised four phenotypically extensive metabolizers (EM)

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