Uptake of [^{14}C]xylocholine in mouse vas deferens after chemical sympathectomy

Amphetamine sulphate has been shown to reverse the adrenergic neuron-blocking action of xylocholine and to displace some 5% of the total tissue content of xylocholine from guinea-pig vas deferens but it is not known if these two observations are causally related, or if the small proportion of the total tissue content of xylocholine displaced is of pharmacological significance. However, a close structural homologue of xylocholine [*NNN*-triethyl-2-(2,6-xylyloxy)- ethylammonium bromide; TE10] which lacks the adrenergic neuron-blocking action of xylocholine is not displaced from vasa deferentia by amphetamine (Dean & Hughes, 1971; 1972).

If only a small proportion of the total tissue content of xylocholine is located in the adrenergic nerves then the observed small displacement of xylocholine by amphetamine might be highly significant. The uptake of xylocholine into normal and sympathectomized vasa deferentia has therefore been compared in an attempt to establish what proportion of the total tissue content of xylocholine is located in the adrenergic nerves. In our hands, surgical sympathectomy of guinea-pig vas deferens (Birmingham, 1970) although effective, produced a weight gain in sympathectomized tissues of some 10–20%. This method of sympathectomy was therefore unsuitable for comparative uptake studies and chemical sympathectomy with 6-hydroxydopamine (6-OHDA: Ralph Emmanuel Ltd) was attempted. Mice were chosen as the experimental animals because of the high cost of this material although it was realized that species variation may complicate comparisons with previous work.

Mature male mice were given 6-hydroxydopamine hydrochloride intravenously through the tail vein both 8 days (2 × 50 mg/kg in 0·9% NaCl and 0·2% ascorbic acid) and 1 day (100 mg/kg similarly) before death. Control and treated animals were killed by cervical dislocation, vasa deferentia were removed, adherent fat and mesentery were dissected away and the tissues were set up in an organ bath at 35·5° in McEwen solution (McEwen, 1956). Contractions of the tissues in response to electrical stimulation (rectilinear pulses, 0·2 ms duration, 40 V, 250 shocks at 50 Hz applied every 5 min through parallel platinum wire electrodes) and to noradrenaline were recorded isotonically (load 200 mg). Vasa deferentia from 6-OHDA treated animals responded to transmural stimulation with significantly smaller contractions ($P < 0{\cdot}001$) than did tissues from control animals and also showed a greatly increased sensitivity to noradrenaline (about 15 fold) although the maximum response which could be elicited from the two groups of tissues was not significantly different ($P > 0{\cdot}1$) (Fig. 1). Catecholamine fluorescence histochemistry by the Falck-Hillarp technique (Falck, 1962) showed a virtual absence of fluorescent nerve terminals in the treated tissues suggesting that a large proportion of the total catecholamine content of the tissues had been removed. The relatively large response to transmural stimulation in the treated animals does not weigh against this suggestion since the concomitant increase in noradrenaline sensitivity could mean that activation of a very few functional nerve fibres would produce a large response from the tissue. Although we have no direct electron microscopic evidence that destruction of adrenergic nerves has occurred, this has been demonstrated in the mouse vas deferens by Furness, Campbell & others (1970) and we would suggest that a similar effect has been produced in these tissues.

Vasa deferentia from control and treated animals were incubated with 2·37 μg/ml [^{14}C]xylocholine for 90 min in McEwen solution at 36° and the [^{14}C] content of the tissues was then determined as described previously (Dean & Hughes, 1971) and expressed as μg xylocholine per g tissue wet weight on the assumption that all ^{14}C in

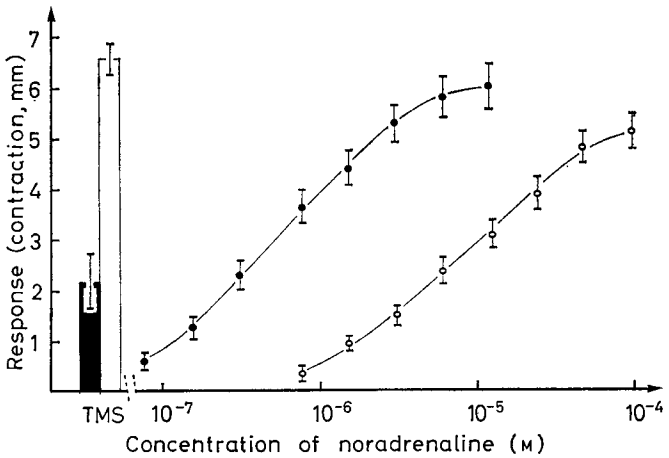

FIG. 1. Showing $\log_{10}$ dose-response curves to noradrenaline and the response to transmural stimulation (TMS) (rectilinear pulses, 0·2 ms duration, 40 V, 50 Hz, 250 shocks every 5 min) in vasa deferentia taken from control (open points; 16 tissues) and 6-hydroxydopamine treated mice (solid points; 10 tissues). McEwen solution, 35·5°, isotonic recording. Responses are shown as mm contraction of the tissue (mean ± standard error).

the tissue was present as xylocholine. (This concentration of xylocholine was chosen since it produces about 90% blockade of the response of the mouse vas deferens to transmural stimulation in 90 min). The tissue content of xylocholine in control and treated vasa deferentia was 26·3 ± 1·2 and 25·6 ± 0·7 μg/g tissue respectively (mean ± standard error; 10 tissues in each group), and represents an approximately 10 fold concentration from the incubation fluid. Although the treated tissues did contain about 3% less xylocholine than did the control tissues this difference was not statistically significant ($P > 0·6$); neither was the difference between the mean weights of the two groups statistically significant ($P > 0·9$).

We would suggest therefore that only a small proportion of the total tissue content of xylocholine is located in the adrenergic nerves where xylocholine must produce its actions and that the displacement of this small amount of xylocholine by amphetamine might be highly significant and account at least in part for the ability of amphetamine to reverse the adrenergic neuron-blocking action of xylocholine.

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