because of mechanical inhibition of isotonic longitudinal muscle shortening through circular muscle activity.

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Analysis of the supersensitivity to noradrenaline induced by amphetamine in the isolated vas deferens of the rat

The development of supersensitivity in sympathetically innervated tissues may be of two different types. One type is specific to noradrenaline or other closely related sympathomimetic amines and dependent on a presynaptic mechanism, probably an impairment of the first step of the uptake process (Trendelenburg, 1963, 1966).

The second type of supersensitivity is non-specific and seems to be linked to a postsynaptic mechanism: modifications in the physiological state of the responding cells (Hudgins & Fleming, 1966; Westfall & Fleming, 1968a, b), or a change in the configuration of the adrenergic receptors (Carrier & Holland, 1965; Varma, 1966; Barnett, Greenhouse & Taber, 1968; Reiffenstein, 1968). Amphetamine is known to inhibit the uptake of noradrenaline (Axelrod, Hertting & Potter, 1962; Burgen & Iversen, 1965; Iversen, 1965, 1967; Häggendal & Hamberger, 1967). Recently it was suggested that amphetamine induces presynaptic supersensitivity to noradrenaline (de Moraes, Carvalho & Wherle, 1970). This report describes an investigation of the specificity of the amphetamine-induced change in sensitivity to noradrenaline.

Vasa deferentia of rats, 200–250 g, were mounted in Krebs-Ringer bicarbonate solution (de Moraes, Carvalho & Wherle, 1970). Dose-response curves to noradrenaline were made on each preparation before and after treatment with amphetamine for 20 min. Horizontal shifts of the log dose-response curves were measured at the level of the EC50. The same general procedure was employed to obtain the dose-response curves to methoxamine. Since it could not be obtained on the same vas deferens, because high concentrations of methoxamine induced spontaneous motility, vasa deferentia from the same rat were used to obtain EC50s. The error introduced is very small as vasa deferentia from the same animal had similar EC50s for methoxamine.

To measure adrenergic blocking activity, the pD'_2 value for noradrenaline-

Table 1. *Comparison of EC50 and maximum contractile response to noradrenaline and methoxamine in isolated rat vas deferens before and after exposure to amphetamine.*

Agonist	Treatment	n	EC50 (Mean ± s.e.)	Maximum contractile response (mm ± s.e.)
Noradrenaline	Control	6	4·733 ± 0·053	31·50 ± 1·52
	Amphetamine (2×10^{-5}M)[a]	5	5·571 ± 0·084[b]	29·75 ± 2·17[c]
	Amphetamine (2×10^{-4}M)[a]	5	6·507 ± 0·050[b]	31·54 ± 1·89[c]
Methoxamine	Control	4	4·767 ± 0·405	24·50 ± 2·66
	Amphetamine (2×10^{-4}M)[a]	4	4·634 ± 0·326[c]	25·75 ± 1·43[c]

n Number of experiments.
EC50 Negative logarithm of the molar concentration producing 50% of the maximum response
[a] Exposure time of 20 min followed by three washes.
[b] Significantly different from control ($P < 0.01$).
[c] Not significantly different from control ($P > 0.05$).

phenoxybenzamine was calculated from the following equation (Bickerton, 1963): $pD'_2 = pD'_x + \log\left[\frac{Eam}{Eabm} - 1\right]$ where pD'_x is the negative logarithm of the molar concentration of phenoxybenzamine which reduced the maximum response to noradrenaline (Eam) to another value (Eabm). Amphetamine was used in "receptor-protection" experiments following the general procedure of Furchgott (1954). After a dose-response curve to noradrenaline was obtained, amphetamine was added to the vas and 20 min later phenxoybenzamine. After 30 min exposure to phenoxybenzamine both drugs were washed out. Post-exposure tests for sensitivity to noradrenaline were made between 60 and 100 min of the beginning of the experiment. The control vas was treated similarly except that amphetamine was absent. The reserpine powder used in these experiments was dissolved in a 20% solution of ascorbic acid and injected 5 mg/kg, i.p. 24 h before the experiment. (−)-Noradrenaline bitartrate, (+)-amphetamine sulphate and (±)-methoxamine hydrochloride were dissolved in distilled demineralized water which contained 0·02 mM of ascorbic acid. Phenoxybenzamine hydrochloride was dissolved in acidified ethanol (Benfey & Grillo, 1963) and diluted in normal saline.

Dose-response curves for noradrenaline and methoxamine obtained before and after exposure of the vas to amphetamine for 20 min followed by three washes were compared. The horizontal shifts of the log dose-response curves measured at the level of EC50 are statistically different only for noradrenaline (*t*-test). The exposure to amphetamine (2×10^{-4}M) for 20 min did not alter the sensitivity of the vas to methoxamine (Table 1).

pD'_2 values for noradrenaline-phenoxybenzamine were determined in another set of experiments. The pD'_2 values, after amphetamine exposure, in animals not pretreated with reserpine are statistically different ($P < 0.05$) from the control value (7·498 ± 0·058; amphetamine: 2×10^{-5}M 6·986 ± 0·032, 2×10^{-4}M 6·942 ± 0·054). However, pre-treatment with reserpine did not affect the pD'_2 values in the absence or in the presence of amphetamine. Also of interest is that pD'_2 value was not altered by the reserpine treatment, in agreement with Green & Fleming (1967).

The evidence presented strongly favours the conclusion that amphetamine induces presynaptic supersensitivity to noradrenaline in the rat isolated vas deferens. Since methoxamine has a direct effect on α-receptors and is not taken up by adrenergic nerve endings (Hertting, 1964) it can be used as an experimental tool to test the role

of the uptake process in the development of the phenomenon of supersensitivity. Amphetamine does not increase sensitivity to methoxamine. This observation is consistent with the view that amphetamine-induced supersensitivity is probably due to an impairment of the uptake mechanism. Baum & Gluckman (1967) reported that amphetamine antagonized the adrenergic blocking activity of phenoxybenzamine in rabbit aortic strips. The pD'_2 values reported here show that the conclusion reached by Baum & Gluckman (1967) who used the rabbit aortic strips, can be applied to the rat vas deferens only if endogenous catecholamines are not first depleted. Amphetamine is an indirectly-acting sympathomimetic amine which releases noradrenaline and simultaneously inhibits the uptake mechanism (Lindmar & Muscholl, 1961, 1965; Iversen, 1967). This fact could explain why, in the vas deferens that has not been pretreated with reserpine, amphetamine seems to antagonize the adrenergic blocking activity of phenoxybenzamine. Perhaps the small amount of noradrenaline released by amphetamine in the non-pretreated preparation, before exposure to phenoxybenzamine, is responsible for the decreased value of pD'_2. On the other hand, the present results seem to exclude a configurational change of the adrenergic receptors to explain the amphetamine-induced supersensitivity.

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