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Effects of denervation, decentralization and cocaine on the responses of the guinea-pig vas deferens to phenylephrine in vivo and in vitro

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Denervation supersensitivity of the guinea-pig isolated vas deferens is the sum of pre- and postjunctional components (Westfall et al 1972). The prejunctional component, which is cocaine-like, results from the impairment of the neuronal uptake process after degeneration of the adrenergic nerve endings (Trendelenburg 1966). The postjunctional component is caused by alterations of the electrophysiological and/or biochemical properties of the smooth muscle cells and is responsible for the non-specific increase in drug sensitivity (Fleming et al 1973; Fleming 1976). It is generally accepted that decentralization supersensitivity of the guinea-pig vas deferens possesses only the postjunctional component (Westfall et al 1972). Our aim has been to compare the effects of denervation, decentralization and cocaine on the responsiveness of the guinea-pig vas deferens to phenylephrine in vivo and in vitro. The evidence confirms the findings of a recent report (Sannomiya & De Moraes 1979) suggesting the absence of the postjunctional component of denervation supersensitivity to noradrenaline in the guinea-pig vas deferens in experiments performed in vivo. In addition, decentralization-induced supersensitivity to phenylephrine was not demonstrable in the guinea-pig vas deferens in vivo.

Guinea-pigs, 350 to 500 g, were anaesthetized with ether and bilateral decentralization was performed by removing 2 cm of the hypogastric nerve (Westfall et al 1972). Unilateral denervation was according to Birmingham (1970). Control animals were sham-operated. In all experiments the animals were used on the 7th day after surgery at a time when postjunctional supersensitivity is fully developed (Westfall 1970).

To assess the effectiveness of the decentralization procedure the animals were anaesthetized with ether and the hypogastric nerves, central to the area of transection, were stimulated (10 Hz, 1 ms duration, 20 V, during 15 s). Any vas deferens that contracted was discarded. To reduce the presence of smooth muscle tonus in the in vivo experiments animals were pretreated with reserpine (2 mg kg^{-1}) and (±)-α-methyl-*p*-tyrosine (250 mg kg^{-1}) 24 and 6 h, respectively, before the experiments began. This pretreatment did not interfere with the response of the vasa deferentia of the control group to electrical stimulation.

Dose-response curves to phenylephrine were obtained in vivo according to Sannomiya & De Moraes (1977). After this has been done, the same vas deferens was isolated and the responsiveness to phenylephrine was again determined, as experiments have shown that the sensitivity of the guinea-pig isolated vas deferens to phenylephrine is not affected by previous exposures to the same agonist in vivo (Sannomiya & De Moraes 1977). Isometric recordings of contractions were made at 37 °C as described by De Moraes (1976) and under a resting tension of 0·5 g. Experiments were also performed using cocaine (5 mg kg^{-1} in vivo and 10^{-5} M in vitro). Changes in sensitivity in vivo or in vitro were evaluated by determining the doses or concentrations of phenylephrine which gave 50% of maximum responses in individual experiments. Mean ED50's or EC50's are presented as geometric means with 95% confidence intervals (Fleming et al 1972). Statistical evaluation comprised Student's *t*-tests for paired and unpaired samples and analysis of variance (Snedecor & Cochran 1967).

Table 1 summarizes the results. Denervation induced a 3·1-fold increase in sensitivity without a significant augmentation of the maximum response to phenylephrine, in experiments in vivo. However, when the responsiveness to phenylephrine was evaluated in vitro, with the same tissues as used in experiments in vivo, there was a 9·3-fold increase in sensitivity without a significant change in the maximum response to the adrenoceptor agonist. On the other hand, cocaine-induced supersensitivity to phenylephrine was 3·8-fold in vivo and 4·3-fold in vitro. Cocaine did not alter the maximum response of the vas to phenylephrine in experiments in vivo or in vitro. In contrast to the results obtained after denervation or cocaine, decentralization did not change the responsiveness of the vas to phenylephrine in vivo. However, after isolation of the same tissues as used in vivo, decentralization caused a small but significant supersensitivity (1·4-fold, $P < 0.05$) without a significant alteration of the maximum response to phenylephrine.

The present experiments confirm that only a cocaine-like denervation supersensitivity to phenylephrine was demonstrable in the vas in vivo. The apparently small increase in sensitivity to phenylephrine after cocaine in vivo and in vitro could be caused by the comparatively low affinity of phenylephrine for the neuronal uptake process (Iversen 1967). On the other hand, it seems plausible to argue that the dose of cocaine, and the denervation procedure used were insufficient to completely impair the neuronal uptake of phenyl-

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Table 1. Effects of denervation, cocaine or decentralization on the responsiveness of the guinea-pig vas deferens to phenylephrine in vivo and in vitro.

Group	$ED50_b$ nmol kg^{-1}	Ratio of ED50's	Maximum response g (s.e.m.)	n_c
$Control_a$	187·9 (61·8–571·1)	—	2·9 (0·4)	25
Denervated	60·0 (25·8–139·5)*	3·1	3·9 (0·5)	7
Cocaine (5 mg kg^{-1})	49·4 (38·0–64·3)*	3·8	3·3 (0·1)	6
Decentralized	226·7 (176·1–273·6)	0·82	2·9 (0·3)	14

Group	$EC50_b$ $\times 10^{-6}$ M	Ratio of ED50's	Maximum response (s.e.m.)$_d$	n_c
$Control_a$	18·7 (14·5–24·2)	—	11·2 (1·4)	25
Denervated	2·0 (0·8–4·7)*	9·3	9·9 (1·1)	7
Cocaine (10^{-5} M)	4·3 (3·6–5·1)*	4·3	9·5 (1·5)	6
Decentralized	12·6 (9·9–16·0)*	1·4	11·6 (0·9)	14

[a] Given is the total number of animals used as a control.
[b] Geometric mean with 95% confidence intervals.
[c] Number of experiments.
[d] g of tension/100 mg wet weight of tissue.
* Significantly different from control ($P < 0.05$).

ephrine in a densely adrenergically innervated tissue such as the guinea-pig vas deferens. However, cocaine-induced supersensitivity to phenylephrine did not differ in vivo and in vitro and a previously published observation showed that 10^{-4} M cocaine produced no greater supersensitivity than did 10^{-5} M in the guinea-pig vas deferens (De Moraes & Capaz 1977). Considering that the sensitizing effect of cocaine was not significantly different in vivo and in vitro, it is possible to assume that the enhanced sensitivity to phenylephrine observed in the denervated vas in vitro was caused by the isolation procedures, i.e. the postjunctional component of denervation supersensitivity was demonstrable only in vitro. It has been reported that there is an 8 to 10 mV depolarization of the smooth muscle cells of denervated or decentralized guinea-pig vas deferens (Fleming & Westfall 1975). This depolarization is probably caused by a denervation or decentralization-induced reduction of activity of an electrogenic Na^+–K^+ pump (Gerthoffer et al 1979).

Probably, this mechanism is only operative when denervated or decentralized vasa deferentia are isolated. Taylor et al (1976) showed that reserpine-induced postjunctional supersensitivity to the rate-increasing effect of drugs in isolated spontaneously-beating atria of the guinea-pigs is dependent on the composition of the bathing medium selected. Decentralization-induced supersensitivity has only the postjunctional component (Westfall et al 1972). Accordingly, we found that decentralization did not induce supersensitivity to phenylephrine in experiments in vivo. There is as yet no explanation for the absence of denervation or decentralization postjunctional supersensitivity to phenylephrine in the guinea-pig vas deferens in vivo. However, the primary importance of this observation is to call attention to the fact that several mechanisms, which have been suggested for the postjunctional component of denervation supersensitivity and/or for decentralization supersensitivity in the guinea-pig vas deferens, could reflect the experimental conditions involved in the isolation of the tissue, not being present in the vas in vivo.

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