Noradrenaline supersensitivity of the mouse vas deferens after long-term treatment with morphine

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It has been reported recently that withdrawal from morphine increases the maximum response of the isolated vas deferens, colon and anococcygeus muscle of the rat without a leftward displacement of dose-reponse curves (Muir & Pollock, 1972; Pollock, Muir & others, 1972; Gardiner, Gibson & Pollock, 1974). Gibson & Pollock (1975) found that morphine withdrawal increased the pD_2 value for acetylcholine on the rat isolated anococcygeus muscle. Collier (1966) proposed a theoretical model to explain dependence on and withdrawal reactions from drugs, based on the development of a specific supersensitivity to an appropriate neurotransmitter. The same author raised the question of whether drug dependence could be induced by an increase in the number of receptors (Collier, 1968). Morphine is known to inhibit noradrenaline release in the transmurally stimulated mouse vas deferens (Hughes, Kosterlitz & Leslie, 1975; Henderson & Hughes, 1976) and prolonged interruption of the contact between a neurotransmitter and its target cells causes supersensitivity (Fleming, McPhillips & Westfall, 1973). In the work presented here we have examined the influence of pretreatment with morphine on the responsiveness of the mouse isolated vas deferens to noradrenaline and potassium. The results clearly indicate that dependence on morphine induces a supersensitivity to noradrenaline probably caused by an alteration in affinity for the adrenoceptors.

Swiss albino mice, 20 to 25 g, at the start of the experiments, were housed in groups of five under a 12 h day–night cycle in an air-conditioned room. The animals were chronically treated with morphine hydrochloride (two daily subcutaneous injections at 8.00 a.m. and 6.00 p.m.) in increasing doses during 10 days according to a previously reported schedule (Kaneto, Koida & others, 1973). Between 1 and 2 h after the last morphine injection, one saline-treated and one morphinized animal were killed by stunning and bleeding and their vasa deferentia set up for organ bath studies. Vasa deferentia were also excised from acutely morphine treated (100 mg kg^{-1}, subcutaneously 1–2 h before killing) and morphine withdrawn mice. The peak intensity of the morphine withdrawal reaction was detected using a behavioral scoring test (Kaneto & others, 1973). The isolated vasa deferentia were set up as previously described by Hughes & Leak (1973). Briefly, the tissues were bathed in McEwen solution (McEwen, 1956) at 36·5°, gassed with 5% carbon dioxide in oxygen and equilibrated under 0·25 g tension for 1 h before any drug testing. Contractions of the vasa were recorded isometrically and displayed on a pen poligraph. Concentration-effect curves to noradrenaline and potassium chloride (K^+) were constructed on tissues isolated from saline-treated, morphine-treated and morphine-withdrawn mice. Noradrenaline was added at 12 min intervals while an 8 min interval was allowed between successive additions of K^+. The concentration-effect curves to K^+ were obtained in the presence of phentolamine (3×10^{-5} M) to reduce the influence of the noradrenaline-releasing effect of the ion (Gibson & Pollock, 1973) and the maximum response to K^+ was determined by replacing the McEwan solution by KCl-Ringer (Edman & Schild, 1962). Horizontal shifts of the concentration-effect curves were measured at the level of the EC50 and presented as dose ratios of geometric means (Fleming, Westfall & others, 1972). To measure adrenoceptor blocking activity, the pD'_2 value for noradrenaline-phenoxybenzamine was calculated from the following equation (Bickerton, 1963):

$$pD'_2 = pD'_x + \log\left[\frac{E_{am}}{E_{abm}} - 1\right]$$

were pD'_x is the negative logarithm of the molar concentration of phenoxybenzamine which reduced the maximum response to noradrenaline (E_{am}) to another value (E_{abm}). The exposure time to phenoxybenzamine was 20 min and the experiments were conducted in the presence of cocaine (10^{-5} M) to increase the accuracy of the estimate (Green & Fleming, 1968).

Table 1 shows the effects of chronic morphine administration, withdrawal from morphine and acute morphine administration on the sensitivity and maximum response of the mouse isolated vas deferens to noradrenaline and to K^+. Tissues isolated from 10-day morphine-treated animals showed an increased sensitivity to noradrenaline, as evidenced by a two-fold leftward displacement of the concentration-effect curve and a greater maximum response to the exogenously added neurotransmitter ($P < 0.05$, t-test). However, vasa deferentia removed from mice at the peak of their morphine withdrawal reaction (which occurred 6 h after the last injection), as previously reported for rat tissues, exhibited only an enhanced maximum response to noradrenaline ($P < 0.05$). A single large dose of morphine did not affect the responsiveness to noradrenaline ($P > 0.05$). No such changes in the responsiveness of the tissue were observed for K^+ in

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Table 1. *Comparison of EC50 and maximum response to noradrenaline and to potassium of the mouse vas deferens after morphine administration and withdrawal.*

Agonist	Group	EC50[a] (95% confidence intervals)	Ratio of EC50's	Maximum response[b] (± s.e.m.)	n[c]
		× 10^{-6} M			
Noradrenaline	Saline-treated	9·15 (7·52–11·13)	—	600·8 ± 45·2	17
	Chronic morphine	4·39 (2·40– 8·06)*	2·08	1021·3 ± 111·3*	9
	Withdrawal	9·23 (4·85–17·56)	0·99	1443·6 ± 160·6*	7
	Acute morphine	8·55 (5·80–12·46)	1·07	575·1 ± 52·2	13
		× 10^{-2} M			
Potassium	Saline-treated	8·40 (7·67– 9·19)	—	1722·9 ± 87·2	20
	Chronic morphine	8·14 (6·59–10·06)	1·03	1844·2 ± 159·0	7
	Withdrawal	7·18 (4·30–11·97)	1·17	1554·2 ± 160·4	5
	Acute morphine	8·72 (7·42–10·25)	0·96	1543·7 ± 121·6	8

[a] EC50, concentration producing a contraction which is 50% of maximum for the agonist in an individual experiment.
[b] mg of tension/10 mg of wet weight of tissue.
[c] n = Number of experiments.
* Significantly different from control value at the 0·05% level of probability.

any of the treatments employed. pD'_2 values for noradrenaline-phenoxybenzamine were determined in another set of experiments using tissues isolated from saline-treated and 10 day morphine-treated mice. A significant increase was observed in the receptor blocking activity of phenoxybenzamine in tissues isolated from morphine dependent animals (saline-treated 8·891 ± 0·087, n = 10 and morphine-treated 9·192 ± 0·101, n = 12, $P < 0·05$, *t*-test).

The evidence presented here favours the conclusion that long-term administration of morphine to mice provokes a supersensitivity to noradrenaline of the vasa deferentia probably through an increase in the affinity of the adrenoceptors. The responsiveness of the tissue to K^+ was not affected in any of the morphine treatments employed suggesting the lack of an unespecific alteration of the effector cells beyond the adrenoceptors. On the other hand, the small but significant shift to the left of the concentration-effect curve to noradrenaline seems to exclude the presence of a prejunctional component as has been described for denervation supersensitivity (Fleming, 1976). Although the pD'_2 value must only be considered as an approximate affinity value, the increased efficiency of the blocking activity of phenoxybenzamine in tissues isolated from morphine-dependent mice suggests that morphine treatment has increased the affinity of the antagonist, and probably of noradrenaline, for the adrenoceptors. However, this subtle increase in the affinity for the adrenoceptors is not a plausible explanation for the enhanced maximum response to noradrenaline induced by chronic morphine administration and withdrawal. An alteration of the relation between activation of the adrenoceptors and the final response, i.e., efficacy (Goldstein, Aronow & Kalman, 1968) could also be involved, as suggested for postjunctional supersensitivity induced by other pharmacological means (Taylor & Green, 1971). The present data are not extensive enough to clarify this possibility. Recently it has been suggested that the factor underlying the augmented maximum response of the rat anococcygeus muscle to noradrenaline and acetylcholine during morphine withdrawal is the raised plasma corticosterone level (Gibson & Pollock, 1975). Nevertheless, the present report suggests that the mouse isolated vas deferens could be a relatively simple and suitable experimental model to study the cellular basis of the dependence to and withdrawal from morphine. The conclusion to be drawn from this communication is that changes in the physiology of peripheral adrenergic neuroeffector junctions could play an important role in morphine dependence and withdrawal.

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REFERENCES

BICKERTON, R. K. (1963). *J. Pharmac. exp. Ther.*, **142**, 99–110.
COLLIER, H. O. J. (1966). *Adv. Drug. Res.*, **3**, 171–188.
COLLIER, H. O. J. (1968). *Nature, Lond.*, **220**, 228–231.
EDMAN, K. A. P. & SCHILD, H. O. (1962). *J. Physiol., Lond.*, **161**, 424–441.
FLEMING, W. W., WESTFALL, D. P., DE LA LANDE, I. S. & JELLETT, L. B. (1972). *J. Pharmac. exp. Ther.*, **181**, 339–345.

FLEMING, W. W., MCPHILLIPS, J. J. & WESTFALL, D. P. (1973). *Ergebn. Physiol.*, **68**, 55–119.
FLEMING, W. W. (1976). *Rev. Neurosci.*, **2**, 43–90.
GARDINER, D. C., GIBSON, A. & POLLOCK, D. (1974). *Life Sci.*, **15**, 339–349.
GIBSON, A. & POLLOCK, D. (1973). *Br. J. Pharmac.*, **49**, 506–513.
GIBSON, A. & POLLOCK, D. (1975). *J. Pharmac. exp. Ther.*, **192**, 390–398.
GOLDSTEIN, A., ARONOW, L. & KALMAN, S. M. (1968). In *Principles of Drug Action*, pp. 70–105. London: Harper & Row.
GREEN, R. D. & FLEMING, W. W. (1968). *J. Pharmac. exp. Ther.*, **162**, 254–262.
HENDERSON, G. & HUGHES, J. (1976). *Br. J. Pharmac.*, **57**, 551–557.
HUGHES, I. E. & LEAK, C. M. (1973). *J. Pharm. Pharmac.*, **25**, 81–82.
HUGHES, J., KOSTERLITZ, H. W. & LESLIE, F. M. (1975). *Br. J. Pharmac.*, **53**, 371–378.
KANETO, H., KOIDA, M., NAKANISHI, H. & SASANO, H. (1973). *Jap. J. Pharmac.*, **23**, 701–707.
MCEWEN, L. M. (1956). *J. Physiol., Lond.*, **131**, 678–689.
MUIR, T. C. & POLLOCK, D. (1972). In: *Agonist and Antagonist Actions of Narcotic Analgesic Drugs*, pp. 207–218. London: McMillan.
POLLOCK, D., MUIR, T. C., MCDONALD, A. & HENDERSON, G. (1972). *Eur. J. Pharmac.*, **20**, 321–328.
TAYLOR, J. & GREEN, R. D. (1971). *J. Pharmac. exp. Ther.*, **177**, 127–135.

Haloperidol inhibits contractions of the vas deferens

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Although the vasa deferentia of several mammalian species contain very high concentrations of noradrenaline (Sjöstrand, 1965; Blakeley, Dearnaley & Harrison, 1970), it is not agreed whether noradrenaline functions as a motor transmitter or whether an as yet unidentified substance performs this function. In the rat and guinea-pig, for example, Ambache and colleagues (Ambache & Zar, 1971; Ambache, Dunk & others, 1972) have provided evidence that adrenoceptor blocking agents do not diminish nerve-mediated contractions of isolated vasa although they do prevent the contractions produced by exogenously applied noradrenaline and by indirectly acting sympathomimetics such as tyramine. Other groups have also noted a large discrepancy between the doses of phentolamine which block noradrenaline and those which reduce electrically-induced contraction (Jones & Spriggs, 1975; Jenkins, Marshall & Nasmyth, 1976). These results have been attributed to a failure of phentolamine to reach a sufficiently high concentration in the narrow extracellular spaces of vasa to inhibit the effects of very high concentrations of synaptically released noradrenaline (Furness, 1974; Jones & Spriggs, 1975).

The present experiments were undertaken to examine the effects of the largely unionized catecholamine antagonist haloperidol on vasa of mice, rats and guinea-pigs. Adult animals were killed by a blow on the head and a vas deferens was immediately removed and placed in cold Krebs solution. The connective tissue and blood vessels were carefully cut away from the vas, which was then suspended in a 25 ml organ bath at 36° containing Krebs solution of the following composition (mM): NaCl, 118; KCl 4·75; $CaCl_2$ 2·54; $MgSO_4$ 1·19; $NaHCO_3$ 25; KH_2PO_4 0·93; glucose 11. The solution was aerated with a mixture of 5% carbon dioxide in oxygen. Contractions were recorded by an isotonic transducer. Stimulation of the intramural nerves was achieved by a pair of ring electrodes around the tissue. The electrodes were 15 mm apart and had diameters of 3 mm. Preparations were usually stimulated to contract with trains of 10 pulses at 50 Hz every 15 or 30 s. The stimuli were of 1 ms duration and were delivered by a Devices Digitimer unit and stimulus isolators.

Drugs were made up in Krebs solution and were usually injected into the bath in volumes of 0·1 ml. The only exceptions were for the higher doses of haloperidol applied to rat and guinea-pig vasa. Haloperidol solutions were made from ampoules of haloperidol for injection (Serenace, Searle). This preparation also contains dextrose and lactic acid, but these were found to have no effect on contractions of vasa. The antagonist was left in contact with vasa for 2 min.

The results are summarized in Fig. 1. Sample records of the effects of haloperidol on electrically-induced and noradrenaline-induced contractions are shown in