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SENSITIVITY OF MOUSE VAS DEFERENS TO NEUROTRANSMITTERS: CHANGES AFTER MORPHINE TREATMENT

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1 The pharmacological responses of the isolated vas deferens of the mouse were investigated after acute and chronic treatment with morphine.

2 The addition of morphine to the bath did not alter the responses of the vas deferens to exogenous noradrenaline, adrenaline or dopamine.

3 Low doses of morphine depressed the responses to acetylcholine. Very high concentrations of the opioid (8.5×10^{-4} M) completely abolished, in about 50% of the preparations, the responses to exogenous acetylcholine, while in the other 50% a potentiation of the responses to low concentrations of acetylcholine was observed.

4 The vas deferens of mice chronically treated with morphine showed increased sensitivity to exogenous noradrenaline, but decreased sensitivity to acetylcholine.

5 A fresh amount of morphine added to the bath enhanced the responses of morphine-tolerant preparations to noradrenaline but not to dopamine or acetylcholine. The specificity of this phenomenon was demonstrated by the use of pentobarbitone instead of the opioid.

6 These results are in agreement with the theory that tolerance could result from a form of disuse supersensitivity.

Introduction

One theory explaining the induction of tolerance to morphine is that put forward by Collier (1965; 1968), which suggests that the analgesic produces its acute effects by reducing the release of neurotransmitter(s). Chronic inhibition causes an increase in the number of active receptors for the transmitter and this results in an attenuation of the effects of the narcotic.

Morphine has been shown to inhibit the release of acetylcholine in the brain (Beleslin & Polak, 1965; Jhamandas, Pinsky & Phillis, 1970; Matthews, Labrecque & Domino, 1973), and also that of noradrenaline in rat cerebral cortex slices (Montel, Starke & Weber, 1974), with the relatively low concentrations needed to produce its pharmacological effects. At the same low concentrations, morphine prevents the contractions of the coaxially stimulated guinea-pig ileum, by inhibiting acetylcholine release (Paton, 1957; Schaumann, 1957; Cox & Weinstock, 1966), and those of the vas deferens of mice, induced by electrical field stimulation, by inhibiting noradrenaline release (Henderson, Hughes & Kosterlitz, 1972; Henderson & Hughes, 1976).

There are other similarities between the actions of narcotic analgesic drugs on the peripheral nervous system and those on the central nervous system. In both systems these effects of morphine can be antagonized by naloxone (Cox & Weinstock, 1966; Matthews *et al.*, 1973; Henderson & Hughes, 1976). The guinea-pig ileum has been shown to exhibit tolerance to the effects of morphine on repeated administration, a characteristic feature of the effect of morphine on the central nervous system (Paton, 1957; Fennessy, Heimans & Rand, 1969; Shoham & Weinstock, 1974).

Furthermore, once chronic morphine administration produces tolerance to the effects of the opioid on the twitch height, there is a concomitant enhancement of the responses to acetylcholine in the ileum (Shoham & Weinstock, 1974). On the other hand, it has been recently reported that long-term administration of the analgesic to mice provokes a supersensitivity to noradrenaline in the vas deferens, probably through an increase in the affinity of the adrenoceptors (Rae, Neto & De Moraes, 1977).

The purpose of the present study was to use the isolated vas deferens of the mouse as a relatively simple and suitable experimental model to study the cellular basis of tolerance to and dependence on morphine.

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Methods

Male mice of a strain raised in this laboratory weighing 25 to 30 g were killed by cervical dislocation; the vas deferens and a small portion of seminal vesicle was dissected out and placed in Tyrode solution. The tissue was mounted vertically, under 0.2 g tension, in a 20 ml organ bath containing Tyrode solution at 30°C.

Drugs and solutions

The bathing fluid was a modified Tyrode solution of the following composition: (mM): NaCl 94, KCl 4.69, $CaCl_2$ 2.52, $MgCl_2$ 0.54, NaH_2PO_4 1.30, $NaHCO_3$ 25.0 and glucose 5.04; it was bubbled with 95% O_2 plus 5% CO_2.

The drugs used were (−)-noradrenaline bitartrate (K & K Laboratories), acetylcholine hydrochloride (Matheson Coleman & Bell), adrenaline hydrochloride (Matheson Coleman & Bell), morphine hydrochloride (May & Baker), neostigmine methyl sulphate (L. Light & Co.), pentobarbitone sodium (May & Baker), 5-hydroxytryptamine (serotonin) creatinine sulphate (Nutritional Biochemicals Corp.), dopamine hydrochloride (Sigma), histamine dihydrochloride (Nutritional Biochemicals Corp.).

Dose-response curves

The preparations were permitted to equilibrate with the Tyrode solution for 30 to 45 min before the addition of any drug. Cumulative dose-response curves of isotonic contractions induced by agonists were obtained by the method described by van Rossum (1963).

To avoid fatigue of the preparation, at least 30 min were allowed to elapse between dose-response curves.

Student's *t* test was used to determine the statistical significance of the difference between the means. All values are the mean ±s.e. mean.

Acute treatment with morphine or pentobarbitone

Two reproducible dose-response curves for each agonist were obtained before any treatment with either morphine or pentobarbitone. Morphine or pentobarbitone was added to the bath and left for 3 min in contact with the preparation before further dose-response curves were obtained.

Chronic treatment with morphine

Mice were chronically treated with morphine over a 3 day period by injection of the following suspension: sorbital sesquioleate 0.8 ml, liquid paraffin 4.2 ml, saline 5.0 ml and morphine (as base) 300 mg (as described by Collier, Francis & Schneider, 1972, but modified by use of sorbital sesquioleate instead of mannide mono-oleate). The volume injected was 0.01 ml/g of body weight. This dose of morphine produces an intense degree of tolerance and physical dependence (Contreras, Tamayo & Quijada, 1977). Mice were injected on the first day and killed on the fourth day.

Some experiments were carried out with mice made tolerant over a 10 day period by injecting them twice with the above suspension (on the first day and on the fourth day), and killed on the eleventh day.

Results

Acute treatment with morphine

The responses of the mouse vas deferens to either noradrenaline or adrenaline were unaffected by morphine (2.65×10^{-5} M to 8.50×10^{-4} M). However, the responses of the vas deferens to acetylcholine were inhibited by concentrations of morphine of 2.65×10^{-5} M, 5.30×10^{-5} M and 1.06×10^{-4} M (Figure 1). The maximum responses of the vas to acetylcholine were more affected than those elicited by low concentrations of the neurotransmitter, but the duration of the contraction was increased at all acetylcholine concentrations. At a morphine concentration of 8.50×10^{-4} M about 50% of the preparations showed an increase in their responses to low doses of acetylcholine (up to 1.0×10^{-4} M) as compared with those in the absence of the opioid. The responses to higher doses of acetylcholine were depressed (Figure 2). In the other 50% of the preparations, this high dose of the narcotic completely abolished the responses to acetylcholine.

When the highest concentration of morphine in the bath (8.50×10^{-4} M) enhanced the responses to low concentrations of acetylcholine, the experiments were repeated in the presence of neostigmine (3.0×10^{-6} M). The potentiation was still seen in the presence of neostigmine. Thus it was unlikely that the potentiation by morphine was due to an anticholinesterase effect at high concentrations.

The contractions of the vas elicited by dopamine were very small, and were not affected by the presence of morphine in the bath. Several experiments were carried out to establish the effect of the acute addition of morphine on the responses of the vas deferens to KCl. Generally, the responses to KCl were variable, and though there seemed to be a trend for the responses to be depressed by morphine we were unable to draw any conclusions about this agent.

The mouse vas deferens did not contract in response to histamine and 5-hydroxytryptamine, even at a concentration as high as 0.2 M in the bath.

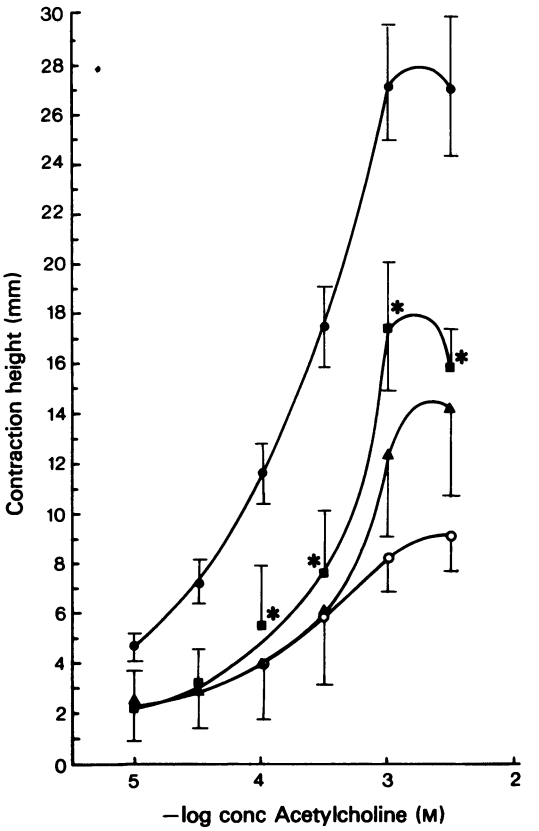

Figure 1 Responses of mouse vas deferens to acetylcholine before, (●, $n = 11$) and after acute treatment with morphine 2.65×10^{-5} M (■, $n = 7$), 5.30×10^{-5} M (▲, $n = 5$) and 1.06×10^{-4} M, (○, $n = 5$). Vertical lines show s.e. mean. *Values statistically different from control values at $P < 0.05$ (unpaired t test).

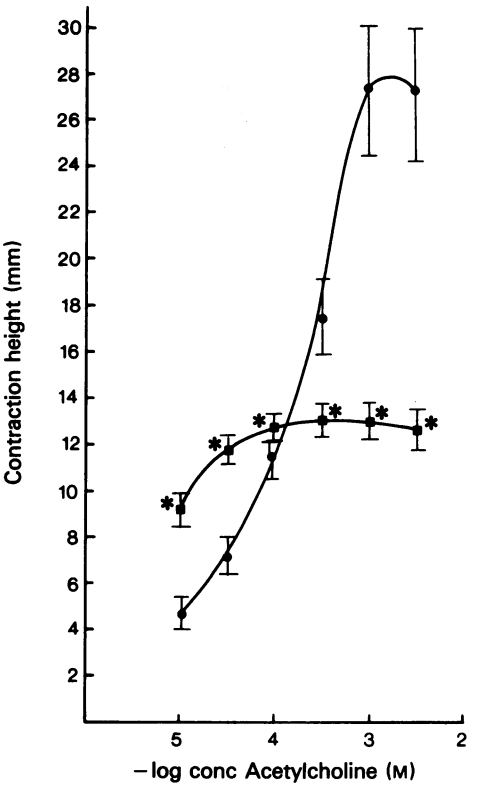

Figure 2 Responses of mouse vas deferens to acetylcholine before, (●, $n = 11$) and after acute treatment with morphine 8.50×10^{-4} M (■, $n = 5$). Vertical lines show s.e. mean. *Values significantly different from control values at $P < 0.025$ (unpaired t test).

Chronic treatment with morphine

Although acute treatment with morphine did not significantly alter the responses of the mouse vas deferens to exogenous noradrenaline, the vas deferens of chronically treated animals showed significantly increased responses to this neurotransmitter as compared with the controls (Figure 3). On the other hand, the response of the mouse vas deferens to acetylcholine was reduced (Figure 4), in contrast to the acute treatment with high doses of morphine (8.50×10^{-4} M) which potentiated the responses to low concentrations of acetylcholine.

The responses to dopamine were not affected by the chronic treatment with morphine.

Dependence on morphine

To determine whether tolerance to morphine was paralleled by a 'dependence' of the vas deferens on the opioid, the preparation from the chronically treated animal was thoroughly washed to decrease the presence of the analgesic and then acutely treated with 1.06×10^{-4} M of morphine. The response to noradrenaline was significantly enhanced in vasa from chronically treated mice in the presence of morphine in the bath as compared with those in the absence of the opioid (Figure 3).

To be sure that this effect was specific to the analgesic, we repeated the experiments replacing morphine in the acute treatment of the mouse vas deferens from chronically treated animals by pentobarbitone. The data show (Figure 5) that pentobarbitone, at several concentrations, depressed the responses of the preparation to noradrenaline, not only in the control animals but also in the morphine-treated ones.

No difference could be observed between the responses to acetylcholine of the chronically treated vas deferens in the absence or presence of morphine in the bath.

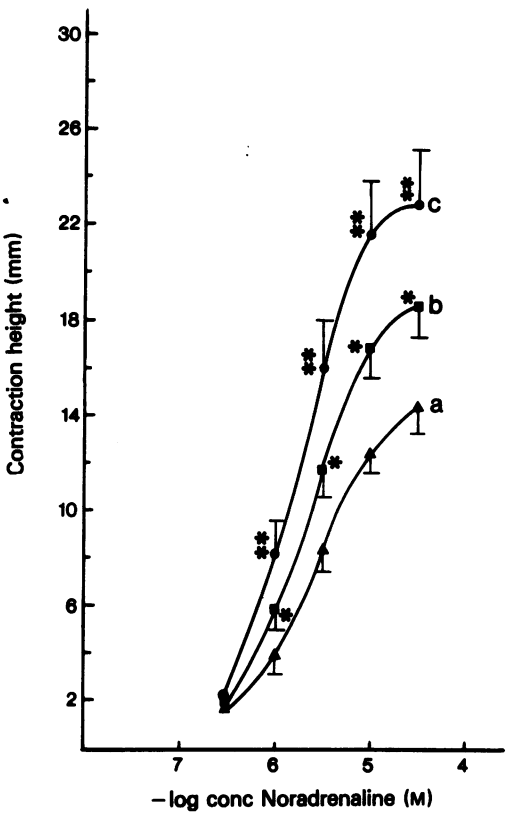

Figure 3 Responses of mouse vas deferens to noradrenaline: (a) untreated mice (▲); (b) 'washed' vas deferens after chronic treatment of mice with morphine (■); and (c) 'washed' vas deferens after chronic treatment of mice with morphine and with acute addition of the analgesic to the bath 1.06×10^{-4} M (●). Mean results from 10 experiments. Vertical lines show s.e. mean. *Values significantly different between (a) and (b) at $P < 0.05$. (unpaired t test); **Values significantly different between (b) and (c) at $P < 0.005$ (paired t test).

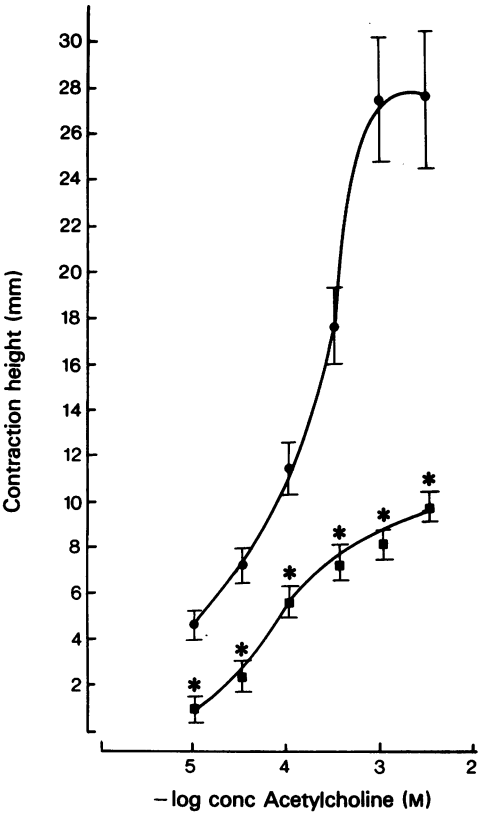

Figure 4 Responses of mouse vas deferens to acetylcholine from animals untreated (●, $n = 11$) and after (■, $n = 11$) chronic treatment with morphine. Vertical lines show s.e. mean. *Values significantly different from control values at $P < 0.005$ (unpaired t test).

Discussion

From these results we infer that morphine not only acts on the nervous structures of the mouse vas deferens but also on the smooth muscle fibres themselves since, as far as we know, there is no evidence for the presence of ganglion synapses within the smooth muscle of the mouse vas deferens or for a cholinergic component in the release of noradrenaline (Henderson & Hughes, 1976); thus the acute treatment with morphine depressed the responses to acetylcholine, an agent that contracts the mouse vas deferens by direct stimulation of the muscle (Henderson *et al.*, 1972). Such a depressant effect of morphine was also deemonstrated by Lewis (1960) in the guinea-pig ileum.

In agreement with the finding of Henderson & Hughes (1976), the acute treatment with the opioid did not reduce the contraction of the mouse vas deferens to either exogenous noradrenaline or to adrenaline. Therefore we may assume that the acute treatment with morphine probably alters some mechanism involved with the contraction in response to acetylcholine but not that involved with the contraction to noradrenaline. Whether this alteration is at the cholinoceptor site or beyond it cannot be concluded from these experiments.

According to Lewis (1960), the effect of morphine (5.30×10^{-7} M) in the guinea-pig ileum preparation was a reduction of about 40% in the response to acetylcholine, with a parallel shift of the whole dose-response curve to the right. No further depression of the dose-response curve could be obtained in this preparation with higher doses of morphine. However, in the mouse vas deferens we found that the depressant effect of morphine was greater at high concen-

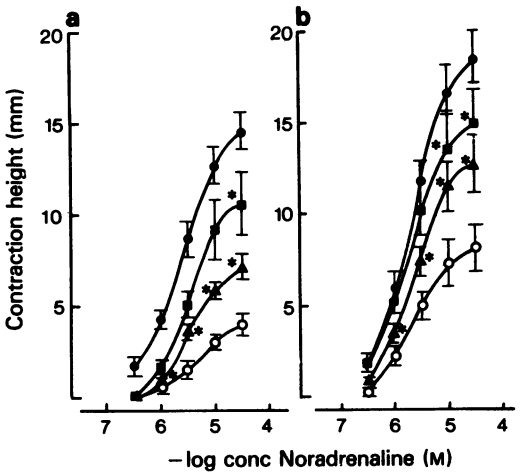

Figure 5 Responses of (a) controls ($n = 12$) and (b) mouse vas deferens chronically treated with morphine ($n = 8$) to noradrenaline in the absence of pentobarbitone (●), in presence of pentobarbitone 0.12 M (■), pentobarbitone 0.24 M (▲) and pentobarbitone 0.36 M (○). Vertical lines show s.e. mean. *Values significantly different from their control values at $P < 0.05$ (paired t test).

trations of acetylcholine than at the lower concentrations, except when very high concentrations of morphine were used, so no parallel shift to the right could be seen although the depression of the contractions was about 36%. Furthermore, increasing morphine concentrations further depressed the maximal responses to acetylcholine (up to 62% and 79% for concentrations of morphine of 5.30×10^{-5} M and 1.06×10^{-4} M respectively) until no responses could be elicited at a concentration of the analgesic of 8.50×10^{-4} M.

In several experiments, the highest concentration of morphine that was used (8.50×10^{-4} M) produced a very peculiar effect characterized by a potentiation of the responses to low concentrations of acetylcholine but an inhibition of the effects of higher concentrations of acetylcholine. At the beginning it was thought that this was an adaptation process of the tissue to morphine, similar to that found by Lewis (1960) in the guinea-pig ileum treated with large doses of morphine, but the experiments with vas deferens from chronically treated mice did not lend support to this explanation. Nonetheless, we cannot disregard the fact that there might be differences between the 'tolerance' induced in the isolated vas deferens from that induced by a chronic treatment with morphine in the whole animals.

A similarity between the chronic effects of morphine on the guinea-pig ileum and the mouse vas deferens lies in the supersensitivity it induces to the contractile action of acetylcholine and noradrenaline respectively. Shoham & Weinstock (1974) demonstrated that the responses of morphine-tolerant ileum to exogenous acetylcholine were enhanced 3 to 10 fold as compared with the non-tolerant strips.

In the present study we found that the tolerant vas deferens became significantly more sensitive to exogenous noradrenaline, possibly as a result of disuse supersensitivity associated with an increase in the affinity of the adrenoceptors (Rae *et al.*, 1977). The morphine-tolerant vas deferens did not show increased sensitivity to acetylcholine. This suggests that sensitization of the vas deferens by morphine may be specific for the 'physiological' neurotransmitter.

Paton (1957) and Lewis (1960) were able to demonstrate that in tolerant guinea-pig ileum, the withdrawal of morphine reduced the response to nerve stimulation and that replacement of morphine restored the response. Since the tolerant mouse vas deferens was permitted to equilibrate for at least 30 minutes with the bath fluid, and that during this period was frequently washed, we considered it to be in a 'withdrawal' state. Under these conditions the fresh addition of morphine significantly enhanced the responses of the strip to noradrenaline. A longer period of chronic morphine treatment of the animal (from 3 to 10 days) did not induce a higher 'dependence' on morphine. This 'dependence on morphine' was apparently specific for the opioid since the addition of pentobarbitone, instead of morphine, to the bath decreased the responses to noradrenaline. Further studies are required to determine the relevance of these effects to the development of 'tolerance' and 'dependence'.

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