Br. J. Pharmac. (1980), **68**, 399–405

THE EFFECT OF CHRONIC MORPHINE TREATMENT ON EXCITATORY JUNCTION POTENTIALS IN THE MOUSE VAS DEFERENS

R.A. NORTH & L.V. VITEK

Neurophysiology Laboratory, Department of Pharmacology, Loyola University Medical Center, Maywood, Illinois 60153, U.S.A.

1 Intracellular recordings were made from smooth muscle cells of vasa deferentia *in vitro*. Vasa from two groups of mice were studied; the first were naive and the second had been chronically pretreated with morphine for 3 days. The vasa from morphine-pretreated mice were maintained in Krebs solution containing normorphine (300 nM).

2 The resting membrane potentials of the smooth muscle cells were the same in both groups of mice.

3 The excitatory junction potentials (e.j.ps) evoked by stimulation of the intramural nerves were depressed by normorphine in both groups of mice. The EC_{50} for this action of normorphine was 560 nM for the naive group and 6.6 μM for the morphine-pretreated group.

4 The EC_{50} for adenosine in depressing e.j.p. amplitude was the same in the two groups.

5 Naloxone did not change the resting membrane potential in cells from either group of mice. In morphine-pretreated mice, naloxone caused a marked increase in the amplitude of the evoked e.j.p.

6 The EC_{50} for noradrenaline in causing a contractile response of the isolated vas deferens was the same in both groups of mice.

7 The results indicate that changes in postsynaptic sensitivity to transmitter do not occur following morphine pretreatment.

Introduction

The contractions of the mouse isolated vas deferens evoked by electrical stimulation of its intramural nerves are inhibited by low concentrations of narcotic analgesics (Henderson, Hughes & Kosterlitz, 1972). This effect is dose-dependent, stereospecific and reversed by the opiate antagonist, naloxone (Hughes, Kosterlitz & Leslie, 1975). Excitatory junction potentials (e.j.ps) can be evoked by nerve stimulation and recorded from single smooth muscle cells of the mouse vas deferens (Holman, 1967). Normorphine depresses the amplitude of the e.j.p. in the mouse vas deferens without affecting the resting membrane properties of the smooth muscle cells (Henderson & North, 1976).

When mice are implanted with morphine pellets for 3 days prior to removal of their vasa deferentia, the stimulus-evoked contractions of their vasa are less sensitive to depression by morphine. However, such tolerance is apparent only if the vasa are maintained in Krebs solution containing opiate drug and declines rapidly and completely by washing the tissue in opiate-free Krebs solution (Cox, 1978). In the present study intracellular recordings were made from smooth muscle cells of vasa taken either from naive mice or from mice pretreated with morphine. The purpose was to determine if morphine pretreatment altered the resting membrane properties of single cells or affected the ability of normorphine to cause acute depression of the amplitude of evoked e.j.ps. A preliminary account of some of these results has been presented (Vitek & North, 1979).

Methods

Intracellular recordings

Male albino mice of the CF-1 strain were killed by a blow to the head; the vas deferens was quickly removed, stripped of all adhering connective tissue and pinned out in a perspex bath. Krebs solution was of the following composition (mM): NaCl 118, KCl 4.75, KH_2PO_4 0.93, $CaCl_2$ 2.54, $MgSO_4 . 7H_2O$ 1.19, glucose 11, $NaHCO_3$ 25 and was gassed with 95% O_2 and 5% CO_2; this solution perfused the preparation at a flow rate of 1.5 ml/min. The temperature at the recording site was 35 to 37°C. Intracellular recordings were made from smooth muscle cells as previously described (Henderson & North, 1976). Drugs were applied by turning a tap which changed the Krebs

0007-1188/80/030399-07 $01.00

solution to another which differed only in its content of drug(s). Values for resting membrane potentials were determined by sudden withdrawal of the recording electrode from the tissue into the perfusion solution.

Intramural nerves were stimulated by two platinum ring electrodes placed concentrically around the muscle on either side of the site of recording. The stimulus was a single rectangular pulse (500 μs or 1 ms in duration); these pulses were repeated at intervals of 30 s. The stimulus voltage was adjusted so that control e.j.ps were between 10 and 25 mV in amplitude before measurement of the acute depressant action of normorphine. These e.j.p. amplitudes were chosen because at lower amplitudes the depressant action of normorphine is less accurately measured and at higher amplitudes non-linear summation (Martin, 1955) may become important. Control e.j.p. amplitudes were usually between 5 and 10 mV before the effect of naloxone on vasa maintained in normorphine (300 nM) was examined.

Contractions of the vas deferens

Contractions of the vas deferens were recorded isometrically by the method of Henderson *et al.* (1972). A single vas deferens was suspended in an organ bath (volume 1.5 ml) through which either drug-free Krebs solution (vasa of naive mice) or Krebs solution containing normorphine (300 nM) (vasa of morphine-pretreated mice) continuously flowed. Noradrenaline doses were given at 12 min intervals. New noradrenaline stock solutions (100 mM) were made daily and contained ascorbic acid (100 μM).

Chronic morphine treatment

Mice of one group (morphine-pretreated) were given nine intraperitoneal injections of morphine sulphate (doses of 40, 60, 80, 100, 120, 140, 140, 140 and 140 mg/kg at 8 h intervals). These animals were killed 2 h after the last dose of morphine and their vasa were rapidly removed and placed in Krebs solution containing normorphine (300 nM). This was an attempt to approximate the *in vivo* condition in which morphine is continually present (Cox, 1978; North & Karras, 1978). Vasa from control (naive) mice, which had received no injections, were placed in drug-free Krebs solution.

Drugs

Drugs used were adenosine (Sigma), (±)-noradrenaline hydrochloride ((±)-arterenol) (Sigma), morphine sulphate (Lilly), naloxone hydrochloride (Endo Laboratories) and normorphine sulphamate (Dr. E.L. May). Concentrations in this paper refer to the final bath concentration of these substances.

Results

Intracellular recordings were made from 72 cells of vasa from 32 naive mice, and from 52 cells of vasa from 16 mice which had been pretreated with morphine. Stable recordings were maintained for up to 3 h, with most successful impalements lasting at least 25 min.

Membrane potentials

The resting membrane potential of vas deferens cells was 62.6 ± 0.9 mV (mean ± s.e. mean, $n = 16$) in naive mice and 63.0 ± 2.1 mV ($n = 11$) in morphine pretreated mice. No obvious differences in the frequency and/or amplitudes of spontaneous e.j.ps were observed between the two groups, although careful quantitative measurements were not made. Normorphine (100 nM to 30 μM) did not alter the membrane potentials of the smooth muscle cells; this confirms the observations of Henderson & North (1976).

Effect of normorphine on the e.j.ps

Normorphine (100 nM to 30 μM) depressed the amplitude of the e.j.p. This effect was independent of the initial e.j.p. amplitude within the range 10 to 40 mV. Composite dose-response curves for depression of e.j.p. amplitude were constructed by pooling data from many cells from both the control and the morphine-pretreated groups (Figure 1). It was not always possible to complete a dose-response curve on a single vas deferens but occasionally impalements were long enough to test all normorphine concentrations, even on a single cell. Morphine pretreatment resulted in a shift of the dose-response curve to the right (Figure 1). The maximum inhibitory effect of normorphine was not altered; depressions of e.j.p. amplitude of 80 to 90% could be obtained in vasa from both naive and morphine-pretreated mice. Also, the depression of e.j.p. amplitude by the highest normorphine concentration tested in naive mice (3 μM) did not differ significantly from the inhibition of the e.j.p. by the highest normorphine concentration tested in morphine-pretreated mice (30 μM). The concentration of drug that decreased the e.j.p. amplitude by 50% (EC_{50}) was 560 nM in naive animals and 6.6 μM in morphine-pretreated animals. Individual EC_{50} values for normorphine in mice pretreated with morphine (3 to 30 μM) did not overlap with individual EC_{50} values in control mice (270 to 890 nM).

It is possible that the shift to the right of the normorphine dose-response curve in vasa from mice that

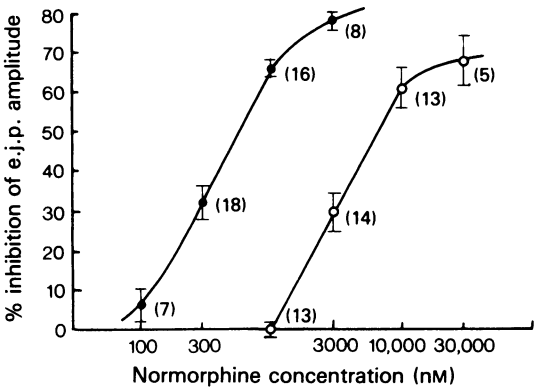

Figure 1 Normorphine dose-response curves for inhibition of evoked excitatory junction potential (e.j.p.) amplitude in the mouse vas deferens. (●) Naive mice; (○) morphine-pretreated mice. Bars represent s.e. mean. Numbers in parentheses indicate the number of individual cells tested at a given concentration. Vasa from morphine-pretreated mice were maintained in Krebs solution containing normorphine (300 nM).

had received chronic morphine treatment was due simply to the presence of normorphine (300 nM) in the perfusion fluid. Therefore, some vasa from naive mice were also maintained in Krebs solution containing normorphine (300 nM) for 1 to 5 h. The effects of normorphine (1 and 3 μM) on e.j.p. amplitude were tested. In these vasa, normorphine (1 and 3 μM) still caused a depression of e.j.p. amplitude. The ability of normorphine (3 μM) to depress e.j.p. amplitude was not different from its action on vasa maintained in drug-free Krebs solution, although normorphine (1 μM) was somewhat less effective (Table 1).

Effect of adenosine on the e.j.p.s

Adenosine depressed the amplitude of the e.j.p.s in vasa deferentia from both control and morphine-pretreated mice without changing the membrane potentials of the smooth muscle cells. This effect was rapid in onset and readily reversed upon washing. The magnitude of the depression of e.j.p. amplitude was related to the dose applied, and the effect did not diminish with repeated applications of the same concentration of adenosine to the same cell. Adenosine was equipotent in vasa from the control and morphine-pretreated groups (Table 2).

Effect of naloxone

It has previously been shown (Henderson & North, 1976) that naloxone (up to 1 μM) does not affect the resting membrane potential or evoked e.j.p. amplitude recorded from single vas deferens cells of naive mice. These results were confirmed in the present study. When naloxone (300 nM) was used to reverse the depression of the e.j.p. caused by application of normorphine (300 nM, for 10 min or less) to vasa from naive mice, the e.j.p. amplitude never increased to more than its control level before administration of normorphine ($n = 8$)(see also Henderson & North, 1976). That is to say, naloxone increased the amplitude of the normorphine-depressed e.j.p. but never more than two fold (the mean depression of e.j.p. amplitude by normorphine (300 nM) was to an amplitude which was 68% of the control amplitude).

In contrast, when vasa were removed from morphine-pretreated mice and maintained in Krebs solution containing normorphine (300 nM), changing to a solution which contained both normorphine and naloxone (300 nM) caused a much larger increase in

Table 1 Depression by normorphine of amplitudes of evoked excitatory junction potentials (e.j.ps) in naive and morphine-pretreated mice

	% inhibition of e.j.p.[1]	
	Normorphine (1 μM)	*Normorphine (3 μM)*
Vasa from control (naive) mice maintained in drug-free Krebs solution	66 ± 1.5 (16)	78 ± 1.8 (8)
Vasa from control (naive) mice maintained in Krebs solution containing normorphine (300 nM)	54 ± 2.1 (8)*	76 ± 1.5 (8)^{NS}
Vasa from morphine-pretreated mice maintained in Krebs solution containing normorphine (300 nM)	0 ± 0.0 (13)*	30 ± 4.3 (14)*

[1] Mean values ± s.e. mean. Numbers in parentheses indicate the number of cells tested at a given concentration. Differences between values from vasa of control (naive) mice maintained in drug-free Krebs solution and values from vasa maintained in normorphine-containing Krebs solution were tested by Student's t test, * $P < 0.01$; NS = $P > 0.05$.

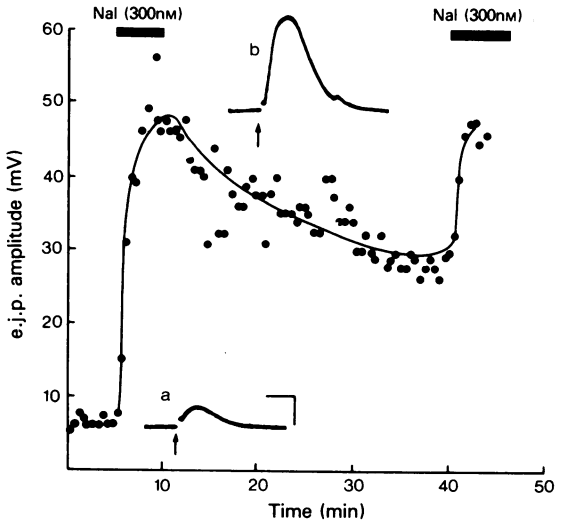

Figure 2 Amplitude of evoked excitatory junction potential (e.j.p.) from a vas deferens of a morphine-pretreated mouse. The perfusing Krebs solution contained normorphine (300 nM) throughout; during the periods indicated by the solid bars, the perfusing solution also contained naloxone (300 nM). Inserts: oscilloscope photographs (partially retouched) of the e.j.p. before (a) and after (b) addition of naloxone. Upward arrows indicate time of stimulus. Insert calibrations: vertical 10 mV, horizontal 100 ms.

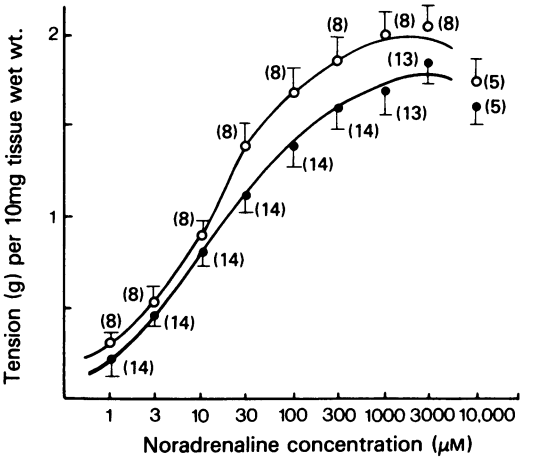

Figure 3 Noradrenaline dose-response curves for the contractile response of the mouse vas deferens. Bars represent s.e. mean. (●) Naive mice; (○) morphine-pretreated mice. Numbers in parentheses indicate the number of preparations tested at a given concentration. The tension produced in response to a given concentration of noradrenaline was significantly different for the morphine-pretreated group only at the concentration of 1 mM ($P = 0.04$).

the amplitude of the e.j.p. In eight cells from eight different preparations, this increase in amplitude was 3.5 to 7 fold (Figure 2). Naloxone did not change the resting membrane potential of smooth muscle cells from vasa of morphine-pretreated mice.

Contractions of vasa deferentia

When noradrenaline (1 μM to 10 mM) was added to the organ bath, the vas deferens showed an increase in tension which peaked rapidly but was not sustained throughout the period of application. The rate of change of tension increased with increasing drug concentration. With high concentrations of noradrenaline (100 μM to 10 mM), the initial peak tension was often followed by a series of rapid fluctuations in tension of lower amplitude; only the initial peak response was measured. Dose-response curves for these responses of the vas deferens to noradrenaline were constructed (Figure 3). The noradrenaline EC_{50} (concentration producing a peak tension which was one-half the maximum response to noradrenaline) was 10.5 μM in controls and 10.4 μM in the morphine-pretreated group. The response to a given concentration of noradrenaline was not significantly different between the two groups of mice except at one dose (1 mM) ($0.01 < P < 0.05$).

The wet weight of the vasa from naive mice (12.7 ± 0.5 mg; mean ± s.e. mean, $n = 14$) did not

Table 2 Depression by adenosine of amplitudes of evoked excitatory junction potentials (e.j.ps) in naive and morphine-pretreated mice

	% *inhibition of e.j.p.*[1]	
	Adenosine (300 μM)	*Adenosine (1 mM)*
Control (naive) mice	42 ± 3.0 (11)	64 ± 3.3 (13)
Morphine-pretreated mice	40 ± 6.0 (8)^{NS}	70 ± 4.4 (9)^{NS}

[1] Mean values ± s.e. mean. Numbers in parentheses indicate the number of cells tested at a given concentration. Differences between values in control (naive) mice and morphine-pretreated mice were tested by Student's t test, NS = $P > 0.05$.

differ significantly from the weight of vasa from mice pretreated with morphine (11.5 ± 0.6 mg, $n = 8$).

Discussion

The three main questions addressed by the present study are as follows. Does chronic morphine pretreatment lead to changes in the resting properties of the smooth muscle cells of the mouse vas deferens? Does tolerance develop to the inhibitory action of morphine at this neuro-effector junction when animals are pretreated with morphine? Can the administration of naloxone *in vitro* to a vas deferens exposed to morphine *in vivo* lead to changes in neuro-effector transmission which are an indication of 'dependence'? These aspects will be discussed in turn.

Resting properties of the smooth muscle cells

Morphine pretreatment did not change the resting membrane potentials of single smooth muscle cells. It has been shown that the smooth muscle cells of the guinea-pig vas deferens become depolarized within a few days of their surgical denervation (Fleming & Westfall, 1975; Fleming, Urquilla, Taylor & Westfall, 1975). Therefore, the effects of morphine pretreatment are not analogous to those of denervation. Presumably, during chronic exposure to morphine *in vivo*, neuro-effector transmission is more or less restored to normality due to the development of tolerance to the initial depressant effect of morphine on transmitter release (see below).

Most of the experimental evidence suggests that noradrenaline is the transmitter released from the sympathetic nerve terminals in the mouse vas deferens that mediates the contractile response of the muscle to transmural nerve stimulation (Jones & Spriggs, 1975; Henderson & North, 1976). Because surgical denervation leads to supersensitivity of smooth muscle to noradrenaline and other substances (*inter alia* Fleming *et al.*, 1975), the effect of chronic morphine treatment on the response of the smooth muscle cells of the mouse vas deferens to exogenously added noradrenaline was examined. We found no change in the sensitivity of the muscle cells to noradrenaline after chronic morphine treatment. Chronic morphine treatment thus differs from surgical denervation in that postsynaptic supersensitivity to transmitter does not develop. Rae, Neto & de Moraes (1977) found that long-term treatment of mice with morphine does lead to a supersensitivity of their vasa deferentia to noradrenaline. They used an intensive schedule for morphine pretreatment which lasted for 10 days and they then placed the isolated tissue in a morphine-free solution. The supersensitivity they observed may, therefore, be a manifestation of the 'withdrawal' state which presumably existed *in vitro*. Such supersensitivity is a common finding in withdrawal from morphine both *in vivo* (Pollock, Muir, MacDonald & Henderson, 1972; Muir & Pollock, 1973; Gibson & Pollock, 1975) and *in vitro* (North & Karras, 1978).

Development of tolerance

Morphine does not block the response of smooth muscle cells of the mouse vas deferens to exogenously applied noradrenaline (Henderson & North, 1976). Morphine does, however, depress noradrenaline release evoked by repetitive nerve stimulation, suggesting that the opiate receptors are located on the intramural nerves (Henderson *et al.*, 1972; Henderson & Hughes, 1976). Thus normorphine depresses the amplitude of the evoked e.j.p. by a prejunctional action on the sympathetic nerve terminals (Henderson & North, 1976).

Twelve times more normorphine was required to depress the e.j.p. in vasa from morphine-pretreated mice than in vasa from naive mice (Figure 1). The isolated tissue may, therefore, be said to be tolerant to the inhibitory action of normorphine on the nerve terminals. Cox (1978) reported a four fold degree of tolerance to the inhibitory action of morphine on the contractions of vasa from mice which had been implanted for 5 days with morphine pellets. In the present experiments, the vas deferens was removed from morphine-pretreated mice and placed in Krebs solution which contained normorphine (300 nM); this was to simulate at least approximately the *in vivo* environment. It may be argued that it was the *in vitro* rather than the *in vivo* exposure to the opiate that induced the observed tolerance. This is unlikely since incubation of vasa from naive mice in normorphine (300 nM) for up to 5 h only slightly changed the sensitivity to normorphine.

The tolerance that develops to the depressant action of normorphine on the e.j.p. may be due directly to the prolonged presence of morphine at the vas deferens *in vivo* or it may be due to an indirect consequence of the morphine pretreatment, such as changes in circulating steroid levels (Gibson & Pollock, 1975). It has been shown that incubation for 24 h of guinea-pig isolated ileum in the presence of an opiate can produce tolerance which is closely similar to that caused by pretreating the guinea-pig with morphine *in vivo* (see reviews by North & Karras, 1978; Cox, 1978). Analogous experiments in which the vas deferens was incubated *in vitro* also demonstrated the induction of tolerance (Henderson & Sim, personal communication).

We sought to demonstrate that morphine pretreatment of mice produced a tolerance that was specific to opiates and not shared by other substances which also depress neuro-effector transmission at this site.

Adenosine inhibits both neurally evoked contractions of the muscle and [^{3}H]-noradrenaline release from the isolated vas deferens of the rat. Furthermore, the responses to exogenous noradrenaline are not affected by adenosine; this indicates a prejunctional mode of action (Clanachan, Johns & Paton, 1977). The effect of adenosine on the e.j.p. in the mouse vas deferens was investigated to determine whether chronic morphine treatment brought about changes in the sensitivity of the intramural nerves to adenosine as well as to morphine. Adenosine depressed the e.j.p. but its ability to do so did not differ between the naive and morphine-pretreated mice (Table 2). That is, morphine pretreatment led to tolerance to the action of morphine without cross-tolerance to adenosine. Similarly, implanting mice with morphine pellets for 3 days does not alter the presynaptic inhibitory effect of the α-adrenoceptor agonist, clonidine (Robson, Gillan, Waterfield & Kosterlitz, 1978).

The effect of naloxone

Naloxone has no effect on the resting membrane potential of the smooth muscle cells or the amplitude of the evoked e.j.p. in vasa from naive mice (Henderson & North, 1976). We found that naloxone also did not change the resting membrane potential of the muscle cells of vasa from morphine-pretreated mice. However, naloxone did cause a large increase in the amplitude of the evoked e.j.p. in such vasa. Was this simply a reversal of the ongoing depression of the e.j.p. by the normorphine present in the bathing solution? Two factors suggest not. First, the vasa should have been tolerant to the normorphine concentration (300 nM) present in the bath fluid since a higher concentration of normorphine (1 μM) did not depress the amplitude of the e.j.p. Second, the increase in e.j.p. amplitude caused by naloxone in the morphine-pretreated group was marked (3.5 to 7 fold). When naloxone was used to reverse the depression caused by a short-lasting (10 min) application of normorphine (300 nM) to vasa from naive mice, the e.j.p. amplitude returned only to its control level before application of drugs. If, however, vasa from naive mice are incubated in normorphine (300 nM) *in vitro* for a longer period of time (5 to 8 h), naloxone causes a larger increase in e.j.p. amplitude (2 to 3 fold) (Vitek & North, unpublished observations). A time-dependent increase in naloxone sensitivity of tissue continuously exposed to normorphine also occurs in the guinea-pig ileum (Schulz & Herz, 1976). In that preparation, a naloxone-precipitated muscle contracture is the manifestation of withdrawal from opiate drugs (see review by North & Karras, 1978). The ability of naloxone to induce a contracture in a tissue removed from a naive guinea-pig increases as the time of exposure to normorphine increases, and this ability is much enhanced if the tissue is taken from a morphine-dependent animal (Schulz & Herz, 1976). In the present experiments, the increase in e.j.p. amplitude caused by naloxone in vasa exposed to normorphine (300 nM) was much greater if the tissue was removed from morphine-pretreated mice as distinct from naive mice. The large increase in e.j.p. amplitude elicited by naloxone in vasa from morphine-pretreated mice may be analogous to the muscle contracture in the guinea-pig ileum. The increase in sensitivity to naloxone of the nerve terminals in the mouse vas deferens may, therefore, represent 'dependence' on morphine in this tissue.

Normorphine inhibits evoked but not spontaneous release of transmitter from the sympathetic nerves in the mouse vas deferens, because the amplitudes of evoked e.j.p.s are depressed (Henderson & North, 1976) but the frequency and amplitudes of spontaneous e.j.p.s are not modified (Henderson, 1976). In the present study naloxone did not cause a massive spontaneous release of transmitter from the nerve terminals of vasa from morphine-pretreated mice. Such a large release would have been observed either as a depolarization of the smooth muscle cells or as a burst of firing of spontaneous e.j.ps: neither occurred. Thus, naloxone-precipitated 'withdrawal' in the mouse vas deferens is manifest as a dramatic increase in evoked release of transmitter (amplitude of evoked e.j.p.) but not as a change in spontaneous transmitter release.

The precise nature of the change in neuro-effector transmission in the mouse vas deferens caused by chronic morphine treatment *in vivo* is not known but it clearly takes place on the nerve terminals which are the site of action of morphine. Tolerance occurs to the usual depressant effects of morphine on transmitter release, and this is not associated with any change in postsynaptic membrane properties or sensitivity to transmitter. This does not support theories of opiate tolerance and dependence which invoke changes in postsynaptic sensitivity following blockade of transmitter release.

This work was supported in part by USPHS grant DA01730 and the Schweppe Foundation. We thank Endo Laboratories and Dr E.L. May for gifts of drugs.

References

CLANACHAN, A.S., JOHNS, A. & PATON, D.M. (1977). Presynaptic inhibitory actions of adenine nucleotides and adenosine on neurotransmission in the rat vas deferens. *Neuroscience*, **2**, 597–602.

COX, B.M. (1978). Multiple mechanisms in opiate tolerance. In *Characteristics and Functions of Opioids* ed. Van Ree, J.M. & Terenius, L., pp. 13–23. Amsterdam: Elsevier.

FLEMING, W.W., URQUILLA, P.R., TAYLOR, D.A. & WESTFALL, D.P. (1975). Electrophysiological correlations with postjunctional supersensitivity. *Fedn Proc.*, **34**, 1981–1989.

FLEMING, W.W. & WESTFALL, D.P. (1975). Altered resting membrane potential in the supersensitive vas deferens of the guinea-pig. *J. Pharmac. exp. Ther.*, **192**, 381–389.

GIBSON, A. & POLLOCK, D. (1975). The involvement of corticosteroids in the supersensitivity produced in the rat anococcygeus muscle by morphine withdrawal, thyroidectomy or a single dose of reserpine. *J. Pharmac. exp. Ther.*, **192**, 390–398.

HENDERSON, G. (1976). Effect of normorphine and enkephalin on spontaneous potentials in the vas deferens. *Eur. J. Pharmac.*, **39**, 409–412.

HENDERSON, G. & HUGHES, J. (1976). The effects of morphine on the release of noradrenaline from the mouse vas deferens. *Br. J. Pharmac.*, **57**, 551–557.

HENDERSON, G., HUGHES, J. & KOSTERLITZ, H.W. (1972). A new example of a morphine-sensitive neuroeffector junction: adrenergic transmission in the mouse vas deferens. *Br. J. Pharmac.*, **46**, 764–766.

HENDERSON, G. & NORTH, R.A. (1976). Depression by morphine of excitatory junction potentials in the vas deferens of the mouse. *Br. J. Pharmac.*, **57**, 341–346.

HOLMAN, M.E. (1967). Some electrophysiological aspects of transmission from noradrenergic nerves to smooth muscle. *Circulation Res.*, **21**, Suppl. 3, 71–82.

HUGHES, J., KOSTERLITZ, H.W. & LESLIE, F.M. (1975). Effect of morphine on adrenergic transmission in the mouse vas deferens. Assessment of agonist and antagonist potencies of narcotic analgesics. *Br. J. Pharmac.*, **53**, 371–381.

JONES, M.E.L. & SPRIGGS, T.L.B. (1975). Noradrenaline and motor transmission in the vas deferens of the mouse. *Br. J. Pharmac.*, **53**, 323–331.

MARTIN, A.R. (1955). A further study of the statistical composition of the end-plate potential. *J. Physiol.*, **130**, 114–122.

MUIR, T.C. & POLLOCK, D. (1973). Morphine-induced changes in the responsiveness of autonomic effector organs. In *Agonist and Antagonist Actions of Narcotic Analgesic Drugs.* ed. Kosterlitz, H.W., Collier, H.O.J. & Villareal, J.E., pp. 207–218. London: MacMillan.

NORTH, R.A. & KARRAS, P.J. (1978). Tolerance and dependence *in vitro*. In *Characteristics and Functions of Opioids.* ed. Van Ree, J.M. & Terenius, L., pp. 25–36. Amsterdam: Elsevier.

POLLOCK, D., MUIR, T.C., MACDONALD, A. & HENDERSON, G. (1972). Morphine-induced changes in the sensitivity of the isolated colon and vas deferens of the rat. *Eur. J. Pharmac.*, **20**, 321–323.

RAE, G.A., NETO, J.P. & DE MORAES, S. (1977). Noradrenaline supersensitivity of the mouse vas deferens after long-term treatment with morphine. *J. Pharm. Pharmac.*, **29**, 310–312.

ROBSON, L.E., GILLAN, M.G.C., WATERFIELD, A.A. & KOSTERLITZ, H.W. (1978). The inhibitory effects of presynaptic α-adrenoceptor agonists on the contractions of the guinea-pig ileum and mouse vas deferens in the morphine-dependent and withdrawn states. In *Characteristics and Functions of Opioids.* ed. Van Ree, J.M. & Terenius, L., pp. 67–68. Amsterdam: Elsevier.

SCHULZ, R. & HERZ, A. (1976). Aspects of opiate dependence in the myenteric plexus of the guinea-pig. *Life Sci., Oxford*, **19**, 1117–1128.

VITEK, L.V. & NORTH, R.A. (1979). Chronic morphine treatment and neuroeffector transmission in the mouse vas deferens. *Fedn Proc.*, **38**, 740.

(Received December 4, 1978.
Revised May 21, 1979.)