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The dependence of excitatory junction potential amplitude on the external calcium concentration in mouse vas deferens during narcotic withdrawal

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1 The dependence of neurotransmitter release on calcium was evaluated in adrenergic terminals from mice that were acutely withdrawn from chronic morphine treatment (CMT).

2 A two fold increase in the number of writhes in response to an i.p. injection of acetylcholine was induced in mice by CMT and subsequent withdrawal.

3 A shift to the left in the relationship between the excitatory junction potential (e.j.p.) amplitude and extracellular calcium concentration ($[Ca]_o$) was induced in vasa deferentia from CMT-withdrawn mice.

4 A reduction in the degree of facilitation of transmitter release during a short low-frequency train of impulses and an increase in the amount of transmitter release during a high-frequency train of impulses was induced in vasa deferentia from CMT-withdrawn mice.

5 The adaptive mechanism of the terminals to the sustained presence of morphine may involve an increase in the probability that the release sites will release transmitter either via increase in calcium influx or an increase in the affinity of calcium to the hypothetical X-receptor.

Introduction

Washed ileum preparations from morphine-tolerant animals showed increased sensitivity to electrical stimulation (Schulz & Cartwright, 1974; Cox, 1978; Collier *et al.*, 1981). Since there is no change in the sensitivity of muscle to transmitter (Ehrenpreis *et al.*, 1975; Frederickson *et al.*, 1976; Johnson *et al.*, 1978), it is likely that the increased response is due to an increase in the quantity of neurotransmitter released by each stimulus in tolerant preparations. When vasa deferentia from mice implanted with morphine pellets were placed in a solution of Krebs containing naloxone, a marked shift to the left of the stimulus-response curve was observed (Illes & Schulz, 1980). These changes suggest that some compensation for the depression in transmitter release induced by the opiates occurs, thereby restoring apparently normal neuronal function. Although the increase in responsiveness of neurones to excitatory stimuli may be mediated via a change in the opiate receptor, there is little evidence for this (Klee & Streaty, 1974; Hölt *et al.*, 1975) and it is more likely that it occurs via a change in the stimulus secretion coupling mechanism, possibly through changes in the calcium-dependent neurotransmitter secretory mechanism.

The calcium-dependence of neurotransmitter release has been studied in the adrenergic nervous system using the mouse vas deferens (Bennett & Florin, 1975) and in the cholinergic nervous system using amphibian skeletal neuromuscular junctions (Dodge & Rahamimoff, 1967; Bennett & Fisher, 1977; Bennett & Lavidis, 1979). During nerve stimulation, the excitability (p) of the terminals in releasing transmitter at skeletal neuromuscular junctions is directly dependent on the extracellular calcium concentration ($[Ca]_o$) i.e. the probability that each terminal will release neurotransmitter when activated by the arrival of an action potential increases with increasing $[Ca]_o$, assuming that not all the release sites recorded from are participating in release (Bennett & Lavidis, 1979; 1982). Although the mouse vas deferens is an electronically coupled syncytium, it is probable that similar dependence of p on $[Ca]_o$ exists at the adrenergic neuromuscular junction in this tissue. In the mouse vas deferens, at any given external calcium concentration morphine reduces the amount of transmitter released during intramural nerve stimulation (Bennett & Lavidis, 1980). It has been shown that the initial inhibition of calcium uptake into release sites by morphine (Lee *et*

Table 1 Morphine dosing schedule

	Group 1 controls (saline ml)		*Group 2* CMT *morphine* ($mg\,kg^{-1}$)		*Group 3* CMT *morphine* ($mg\,kg^{-1}$)		*Group 4* CMT *morphine* ($mg\,kg^{-1}$)	
	0600 h	*1800 h*	*0600 h*	*1800 h*	*0600 h*	*1800 h*	*0600 h*	*1800 h*
Day 1	(Writhing test at 0500 h)							
	0.1	0.1	10	10	10	10	10	10
Day 2	0.1	0.1	10	10	10	10	10	10
Day 3	0.1	0.1	10	10	10	30	10	30
Day 4	0.1	0.1	10	10	30	30	30	30
Day 5	0.1	0.1	10	10	30	30	100	100
Day 6	(Writhing test at 0900 h, morning morphine dose delayed to 1000 h)							
	0.1	0.1	10	10	30	30	100	100
Day 7 to Day 10	0.1	0.1	10	10	30	30	100	100
Electrophysiological studies								

al., 1975; Cardenas & Ross, 1976; Harris *et al.*, 1977; Yamamoto *et al.*, 1978; Guerrero-Munoz *et al.*, 1978) is reversed during tolerance and enhanced during withdrawal (Yamamoto *et al.*, 1978). In this study the dependence of neurotransmitter release on calcium was evaluated in adrenergic terminals from mice that were acutely withdrawn from chronic morphine treatment.

Methods

Tissue preparation

Balb C strain male mice were used. Four groups of twelve animals each received either physiological saline (0.1 ml) or morphine hydrochloride, 10, 30 or 100 $mg\,kg^{-1}$ in 0.1 ml saline (s.c.) twice daily for ten days, according to the schedule in Table 1. On the first and sixth day of treatment all groups were tested for nociception using the writhing test (Collier *et al.*, 1968). On the sixth day the test was conducted 15 h after the last morphine dose. On the 10th day of morphine treatment the animals were injected with the scheduled morphine dose, 5–6 h later were killed by a cervical fracture and both vasa deferentia removed and prepared as previously described (Einstein & Lavidis, 1984). In all experiments the isolated vas deferens was placed in a modified Krebs solution containing (mM): Na^+ 141, K^+ 4.7, Mg^{2+} 1.2, Cl^- 157, $H_2PO_4^-$ 1.3, SO_4^{2-} 1.2, HCO_3^- 14.3, and glucose 7.8 and the lowest extracellular calcium concentration to be used (0.7 mM). $[Ca]_o$ was increased by increasing the amount of $CaCl_2$ dissolved in the Krebs solution supplying the bath. The temperature in the bath was maintained between 33 and 35°C.

Stimulation and recording

Intramural sympathetic nerves were stimulated with 2 platinum ring electrodes placed around the vas deferens and about 1 mm apart. Single impulses of 60 V and 0.05 ms duration were used to reduce the need for corrections due to nonlinear summation (Martin, 1955). Over 86% of the excitation junction potential (e.j.p.) amplitudes were less than 12 mV. The error due to nonlinear summation was therefore less than 11% and no correction was made since such a small error does not affect the main conclusions. A minimum interval of 30s between impulses and trains of impulses was allowed (Bennett, 1973).

Intracellular potentials were recorded from smooth muscle cells with glass microelectrodes filled with 2M KCl and having resistances of 50–90 MΩ. The signals were passed through a high impedance unity gain amplifier and photographed from an oscilloscope display. The quality of intracellular impalement was judged to be adequate if there was a negative shift of at least 50 mV in the recording and if miniature excitatory junction potentials (m.e.j.ps) were present throughout the recording period.

Facilitation is defined as e.j.p. (test impulse) divided by e.j.p. (conditioning or first impulse in a train) and was measured and quantified as described previously (Einstein & Lavidis, 1984).

Results

Effect of withdrawal on the writhing response of mice to an i.p. injection of acetylcholine.

In control mice an i.p. injection of acetylcholine ($3.2\,mg\,kg^{-1}$) caused an average of 2 writhes per min. After 5 days of chronic morphine treatment (CMT)

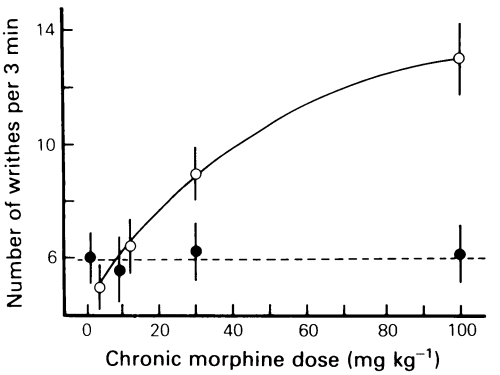

Figure 1 Effect of acute morphine withdrawal on the response of mice to an i.p. injection of acetylcholine (Collier *et al.*, 1968). (●) All groups on day 0, (○) all groups on the 6th day of chronic morphine treatment (CMT), 15 h after the last morphine injection. Each point shows the mean and vertical lines s.e. mean.

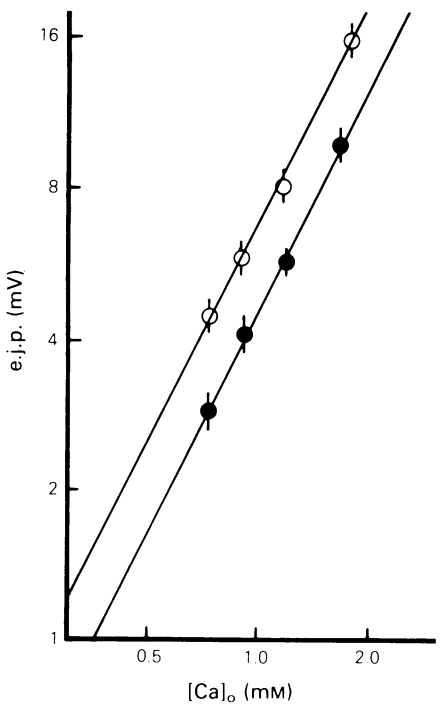

Figure 2 Effect of acute morphine withdrawal on the relationship between transmitter release (measured as e.j.p. amplitude, Burnstock & Holman, 1961) and extracellular calcium. (●) Controls, (○) chronic morphine treatment (CMT 100 mg kg^{-1}) followed by acute withdrawal. Experimental data ($n > 48$) shown are means and vertical lines s.e.mean; lines were fitted using equation 1.

followed by acute withdrawal there was a period of marked inactivity accompanied by an increased sensitivity of the mice to the injection procedure. When these mice were tested for writhing there was an increase in the number of writhes, which varied with the dose of morphine, such that the high CMT dose group (100 mg kg^{-1}) showed a greater than two fold increase in the number of writhes (Figure 1).

Dependence of the e.j.p. amplitude of the mouse vas deferens during withdrawal on [Ca]$_o$

Supersensitivity was also observed in the responses of vas deferens release sites from tolerant, withdrawn animals to single impulse electrical stimulation. A 66% increase in the amplitude of e.j.ps was observed at any given [Ca]$_o$. This increase in sensitivity of the release sites to [Ca]$_o$ was reflected by a shift of the e.j.p. vs [Ca]$_o$ relationship to the left, without any change in the power relationship (Figure 2). The change occurred without any increase in the maximum e.j.p. amplitude that could be recorded, since the y-intercept of the double reciprocal plot of amount of transmitter released vs [Ca]$_o$ was unaltered. Only the K[Ca]$_o$ values were different i.e. 1.39 mM and 1.04 mM [Ca]$_o$ in control and withdrawn terminals respectively (Figure 3). The resting membrane potential of the smooth muscle cells was not

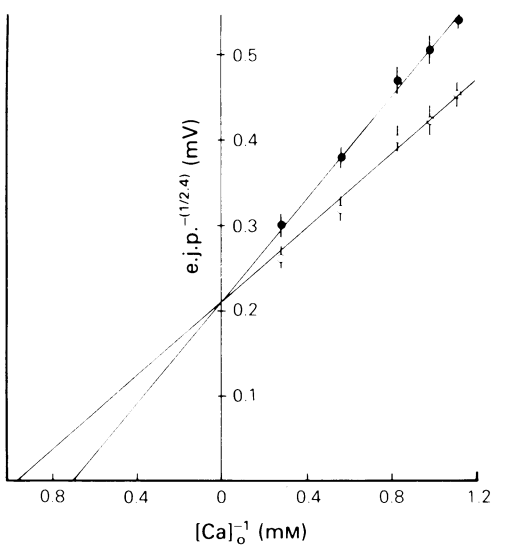

Figure 3 A double reciprocal plot of the amount of transmitter released vs extracellular calcium in control and acutely withdrawn mice. (●) Controls, (○) acutely withdrawn from chronic morphine treatment (CMT; 100 mg kg^{-1}). Mean and s.e.mean (vertical lines) of experimental results ($n > 48$) are shown. E.j.p. (max) = 42 mV and K[Ca]$_o$ values were 1.39 mM for controls and 1.04 mM for withdrawn mice.

altered by CMT nor did the amplitude of miniature e.j.ps change during this period. Using the experimentally derived values for e.j.p. (maximum) and the appropriate $K[Ca]_o$ values it was possible to predict the behaviour of the terminal to a wider range of $[Ca]_o$ using the following equation: (Dodge & Rahaminoff, 1967; Bennet & Lavidis, 1979).

$$\text{e.j.p.} = \text{e.j.p.}_{(max)} \left\{ \frac{[Ca]_o}{K[Ca]_o + [Ca]_o} \right\}^{2.4}$$

where $K[Ca]_o$ is a complex constant.

Changes in facilitation of transmitter release during low frequency stimulation of the mouse vas deferens following withdrawal

Following a conditioning impulse the decay of facilitation of transmitter release by a test impulse was unchanged by CMT (Figure 4). The time constant of facilitation decay remained at approximately 6 s. Increasing the $[Ca]_o$ from 1.2 mM to 1.8 mM decreased the degree of facilitation of transmitter release. CMT and subsequent withdrawal produced a decline in the degree of facilitation at both of these $[Ca]_o$ (Figure 5). As the $[Ca]_o$ of both the control and CMT vasa deferentia was increased, there was a gradual increase in the amplitude of the first impulse and therefore a reduction in the amount of facilitation of transmitter release that could occur (Figure 5). The first e.j.p. was always greater for the acutely withdrawn vasa deferentia than for the controls at any calcium concentration (0.7 to 1.8 mM) and consequently facilitation was reduced (Figure 5). At a $[Ca]_o$ of 1.8 mM very little facilitation in the control vasa deferentia was observed and in the CMT withdrawn vasa deferentia it was common to see spikes on the e.j.ps and depression by the fourth or fifth e.j.p. during a short low frequency train of impulses. Less than 14% of the e.j.ps studied attained values greater than 12 mV. Half of these attained e.j.p. amplitudes which resulted in spikes. No correction for nonlinear summation was carried out since such a small error does not significantly affect the main conclusions. Similar effects could be induced in control vasa deferentia by increasing the $[Ca]_o$. Using the time constant of decay of facilitation of transmitter release (Figure 4) and the residual calcium receptor hypothesis (Bennett & Fisher, 1977) it was possible to estimate accurately the degree of facilitation of every impulse in the train (assuming there was very little depression) in both the control and withdrawn vasa deferentia (Figure 6).

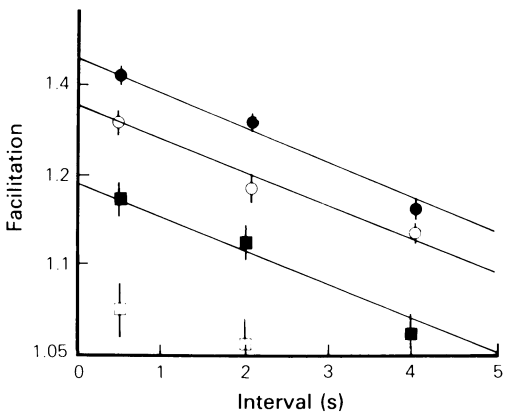

Figure 4 A comparison of the time course of facilitation decay of a conditioning impulse following a test impulse between control and acutely withdrawn (AW) terminals. (●) Controls, 1.2 mM $[Ca]_o$; (○) AW, 1.2 mM $[Ca]_o$, (■) controls, 1.8 mM $[Ca]_o$ and (□) AW, 1.8 mM $[Ca]_o$. Experimental data ($n > 56$) shown as means and vertical lines s.e.mean; lines fitted using linear regression with coefficients > 0.92. The time constant of facilitation decay was approximately 6 s in all lines.

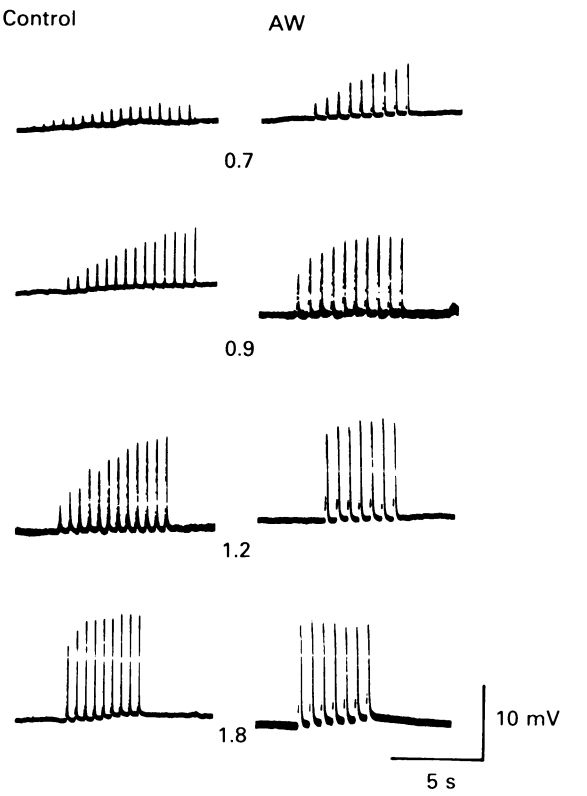

Figure 5 Recordings of the facilitation in transmitter release during low frequency stimulation in controls (left) and acutely withdrawn (AW; right) vasa deferentia. Numbers between recordings indicate the $[Ca]_o$ (mM).

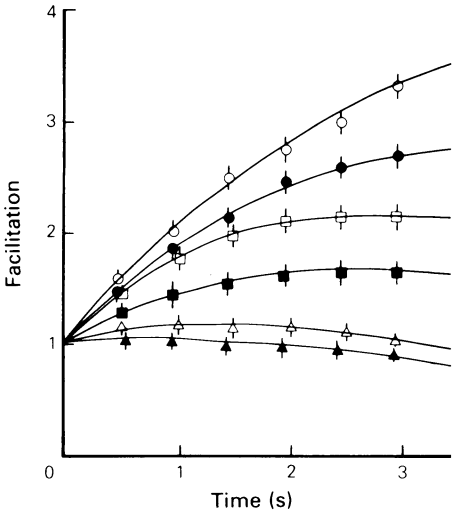

Figure 6 Effect of acute withdrawal (AW) on the facilitation observed during short (2 Hz) train of impulses at various extracellular calcium concentrations. (○) Controls, 0.9 mM $[Ca]_o$; (□) controls, 1.2 mM $[Ca]_o$; (△) controls, 3.6 mM $[Ca]_o$; (●) AW, 0.9 mM $[Ca]_o$; (■) AW, 1.2 mM $[Ca]_o$; (▲) AW, 3.6 mM $[Ca]_o$. Experimental data ($n > 36$) are shown as means and vertical lines represent s.e.mean; lines were fitted using the residual calcium receptor hypothesis.

Depression of transmitter release during high frequency stimulation of the mouse vas deferens following withdrawal

During a high frequency train of impulses, in a high $[Ca]_o$, there was an increase in the amplitude of the e.j.p. Facilitation of transmitter release either reached a steady state or declined to a depressed steady state. In comparison, CMT withdrawn preparations showed less facilitation in transmitter release with a greater degree of depression and a more depressed steady state than the controls (Figure 7). In a $[Ca]_o$ of 0.9 mM control vasa deferentia facilitated to a steady state, CMT withdrawn vasa deferentia in the same $[Ca]_o$ showed less facilitation reaching a steady state which was less than the control. At a higher $[Ca]_o$ (3.6 mM), control animals showed very little facilitation of transmitter release with a prominent depression in transmitter output followed by a depressed steady state. In comparison CMT withdrawn preparations showed less facilitation in transmitter release with a greater degree of depression and a more depressed steady state in transmitter release than the controls (Figure 7). At all extracellular calcium concentrations used the degree of depression in transmitter release was greater in the CMT withdrawn vasa deferentia, the magnitude of these differences decreased with increasing $[Ca]_o$.

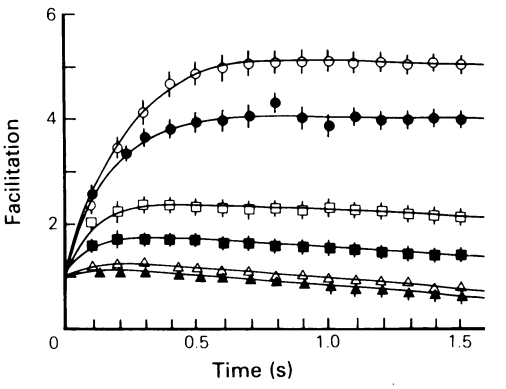

Figure 7 Responses of the terminal to high frequency (10 Hz) train of impulses in control and acutely withdrawn (AW) animals. (○) Controls, 0.9 mM $[Ca]_o$; (□) controls, 1.2 mM $[Ca]_o$; (△) controls, 3.6 mM $[Ca]_o$; (●) AW, 0.9 mM $[Ca]_o$; (□) AW, 1.2 mM $[Ca]_o$; (▲) AW, 3.6 mM $[Ca]_o$. Experimental data ($n > 36$) show means and vertical lines s.e.mean.

Discussion

Changes in the writhing response of mice to an i.p. injection of acetylcholine during acute morphine withdrawal

It is generally accepted that chronic morphine treatment initiates adaptive mechanisms within a cell which can be measured when morphine is acutely withdrawn. The tissues of animals acutely withdrawn from such tolerant states show an increased responsiveness to excitatory stimulation (Pollock *et al.*, 1972; Muir & Pollock, 1973). The two fold increase in the number of writhes produced by an i.p. injection of acetylcholine indicates that the dosage schedule used in these experiments did induce a state of narcotic tolerance and increased responsiveness on withdrawal.

The dependence of e.j.p. amplitude on $[Ca]_o$ in mouse vas deferens during acute withdrawal

Neuromuscular preparations isolated from animals rendered tolerant by repetitive morphine dosing and placed in morphine-free bathing solutions were shown to be more sensitive to electrical stimulation (Schulz & Cartwright, 1976; Cox, 1978), prostaglandin E_1, (Schulz & Cartwright, 1974) and 5-hydroxytryptamine (Schulz & Goldstein, 1973). Illes & Schultz (1980) showed that addition of naloxone to vasa deferentia continuously exposed to morphine resulted in a large parallel shift to the left of the stimulus vs e.j.p. curve. In the present study, a similar shift to the left in the relationship between the e.j.p.

amplitude and $[Ca]_o$ was also shown i.e. the release sites were more sensitive to $[Ca]_o$. This change occurred without any change in the power relationship indicating that the dependence of the stimulus secretion coupling on $[Ca]_o$ was unchanged.

Generally the amount of transmitter that is released following an impulse is a product of two components, the number of terminals in the vicinity of the recording electrode and the average probability of these terminals in releasing transmitter. Using a double reciprocal plot of e.j.p.$^{1/2.4}$ against $[Ca]_o$ it was shown that the maximal e.j.p. amplitude that could be recorded was unchanged in the withdrawn vasa deferentia. $K[Ca]_o$ is a complex constant involving the entry of Ca^{2+} and its actions within the terminal in promoting transmitter release. The probability of transmitter release is related to $K[Ca]_o$. The reduction in the $K[Ca]_o$ induced by CMT and withdrawal caused an increase in probability and so the amount of transmitter released was increased for any given $[Ca]_o$ (0.7 to 1.8 mM). The probability of transmitter release also increases during a train of impulses leading to facilitation of transmitter release (Bennett & Lavidis, 1979).

The effect of acute withdrawal from morphine on the release of transmitter during low frequency trains of impulses

In control preparations, increasing the $[Ca]_o$ increases the probability of transmitter release and this leads to a decrease in the degree of facilitation (Bennett & Lavidis, 1982). Acute withdrawal from CMT produced a marked increase in transmitter release probability with e.j.p. (max) remaining constant; this leads to a reduction in the facilitation of transmitter release during a train of impulses. This reduction in facilitation during a short, low frequency train of impulses at any given $[Ca]_o$ could be reproduced by increasing $[Ca]_o$ and may be due to either an increase in the influx of calcium into the release sites following activation of the terminal by an impulse, or an increase in the binding affinity of calcium for the hypothetical X-receptor or by a reduction in the sequestering of Ca^{2+} within the release sites. Any change in the rate of Ca^{2+} sequestering within the terminal is unlikely, since the time constant of facilitation decay remained constant (6 s) in the acutely withdrawn vasa deferentia. The increase in excitability was so large that during a short train of impulses in 1.8 mM $[Ca]_o$ it was common (7%) to record spikes on top of the e.j.ps with subsequent contraction of the smooth muscle cells. This was never observed in the control vasa deferentia at this $[Ca]_o$ with the stimulus parameters used. A similar increased excitability of neurones during withdrawal has been described in the guinea-pig ileum (Schultz & Herz, 1976; Cox & Padhya, 1977; Collier *et al.*, 1981), vas deferens (Illes & Schulz, 1980) and myenteric neurones (Takagi *et al.*, 1965; Schulz & Goldstein, 1973; Schulz *et al.*, 1974; Ward & Takemori, 1976; Johnson *et al.*, 1978).

The effect of acute withdrawal from morphine on the release of transmitter during high frequency trains of impulses

Following the small amount of facilitation in transmitter release during high frequency trains of impulses, a gradual, $[Ca]_o$-dependent depression of transmitter output was observed. This depression in transmitter release during the last few impulses in a train may be due either to a depletion of transmitter available for release or to transmitter mediated autoinhibition (Bennett & Fisher, 1977; Bennett & Lavidis, 1979; 1982). CMT and acute withdrawal produced a marked increase in the depression of transmitter release during the last few impulses in a high frequency train at every $[Ca]_o$ tested. It is possible that the increase in excitability which occurs in acutely withdrawn vasa deferentia results in a greater release of transmitter during the first few impulses of the train. This is followed by either a greater depletion of available transmitter or a greater autoinhibition which enhances the depression of transmitter release in much the same way as an increase in $[Ca]_o$ would.

The marked reduction in the excitability of nerve release sites and the consequent reduction in transmitter release by acute morphine is achieved either by a potassium-dependent hyperpolarization of the nerve release sites and an indirect reduction in the amount of calcium entering the release sites (Illes & North, 1982; Morita & North, 1982), by direct action on the mechanism involved with calcium entry or by an action on the linking of calcium to the secretory mechanism (Bennett & Lavidis, 1980). Chronic exposure of release sites to morphine results in an adaptive response in which the excitability of the release sites is returned towards the control by the reversal of the above (Einstein & Lavidis, 1984).

Acute withdrawal from morphine unmasks the adaptive response which is observed as a marked increase in e.j.p. amplitude and depression, and a marked decrease in facilitation of transmitter release during low and high frequency trains of impulses, making it unlikely that the adaptive changes are due to any changes on the postsynaptic membrane. Furthermore, the resting potential of smooth muscle cells and the amplitude of miniature e.j.ps of withdrawn cells were not different from those of controls (North & Vitek, 1980). The adaptive mechanism of

the release sites to the sustained presence of morphine therefore involves an increase in the ability of the nerve release sites to release transmitter either via an increase in calcium influx or an increase in the affinity of calcium for the hypothetical X-receptor.

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References

BENNETT, M.R. (1973). An electrophysiological analysis of the storage and release of noradrenaline at sympathetic nerve terminals. *J. Physiol.*, **229,** 515–531.

BENNETT, M.R. & FISHER, C. (1977). The effect of Ca ions on the binomial parameters that control acetylcholine release during trains of nerve impulses at amphibian neuromuscular synapses. *J. Physiol.*, **271,** 673–698.

BENNETT, M.R. & FLORIN, T. (1975). An electrophysiological analysis of the effects of Ca ions on neuromuscular transmission in the mouse vas deferens. *Br. J. Pharmac.*, **55,** 97–104.

BENNETT, M.R. & LAVIDIS, N.A. (1979). The effect of Ca ions on the secretion of quanta evoked by an impulse at nerve terminal release sites. *J. gen. Physiol.*, **74,** 429–456.

BENNETT, M.R. & LAVIDIS, N.A. (1980). An electrophysiological analysis of the effects of morphine on the calcium dependence of neuromuscular transmission in the mouse vas deferens. *Br. J. Pharmac.*, **69,** 185–191.

BENNETT, M.R. & LAVIDIS, N.A. (1982). Vasiation in quantal secretion at different release sites along developing and mature motor terminal branches. *Develop. Brain Res.*, **5,** 1–9.

BURNSTOCK, G. & HOLMAN, M. (1961). The transmission of excitation from autonomic nerve to smooth muscle. *J. Physiol.*, **155,** 117–133.

CARDENAS, H.L. & ROSS, D.H. (1976). Calcium depletion of synaptosomes after morphine treatment. *Br. J. Pharmac.*, **57,** 521–526.

COLLIER, H.O.J., CUTHBERT, N.J. & FRANCIS, D.L. (1981). Model of opiate dependence in the guinea-pig isolated ileum. *Br. J. Pharmac.*, **73,** 921–932.

COLLIER, H.O.H., DINNEEN, L.C., JOHNSON, C.A. & SCHNEIDER, C. (1968). The abdominal constriction response and its suppression by analgesic drugs in the mouse. *Br. J. Pharmac.*, **32,** 295–310.

COX, B.M. (1978). Stimulus-response relationships in ileum preparations from normal and morphine treated guinea-pigs. *Br. J. Pharmac.*, **62,** 387–388.

COX, B.M. & PADHYA, R. (1977). Opiate binding and effect in ileum preparations from normal and morphine pretreated guinea-pigs. *Br. J. Pharmac.*, **61,** 271–278.

DODGE, F.A. & RAHAMINOFF, R. (1967). Co-operative action of calcium ions in transmitter release at the neuromuscular junction. *J. Physiol.*, **193,** 419–432.

EHRENPREIS, S., GREENBERG, J. & COMATY, J.E. (1975). Mechanism of development of tolerance to injected morphine by guinea-pig ileum. *Life Sci.*, **17,** 49–54.

EINSTEIN, R. & LAVIDIS, N.A. (1984). The dependence of excitatory junction potential amplitude on the external calcium concentration in narcotic tolerant mouse vas deferens. *Br. J. Pharmac.*, **83,** 853–861.

FREDERICKSON, R.C.A., HEWES, C.R. & AIKEN, J.W. (1976). Correlation between the *in vivo* and an *in vitro* expression of opiate withdrawal precipitated by naloxone: their antagonism by 1-(−)-Δ^9 tetrahydrocannabinol. *J. Pharmac. exp. Ther.*, **199,** 375–384.

GUERRERO-MUNOZ, F., CERRETA, K.V., GUERRERO, M.L. & WAY, E.L. (1978). Effect of morphine on synaptosomal Ca uptake. *J. Pharmac. exp. Ther.*, **209,** 132–136.

HARRIS, R.A., YAMAMOTO, H., LOH, H.H. & WAY, E.L. (1977). Discrete changes in brain calcium with morphine analgesia, tolerance-dependence and abstinence. *Life Sci.*, **20,** 501–506.

HÖLT, V., DUM, J., BLÄSIG, J., SCHUBERT, P. & HERZ, A. (1975). Comparison of *in vivo* and *in vitro* parameters of opiate receptor binding in naive and tolerant/dependent rodents. *Life Sci.*, **16,** 1823–1828.

ILLES, P. & NORTH, R.A. (1982). Effects of divalent cations and normorphine on spontaneous excitatory junction potentials in the mouse vas deferens. *Br. J. Pharmac.*, **75,** 599–604.

ILLES, P. & SCHULZ, R. (1980). Inhibition of neuroeffector transmission by morphine in the vas deferens of naive and morphine-treated mice. *Br. J. Pharmac.*, **71,** 195–200.

JOHNSON, S.M., WESTFALL, D.P., HOWARD, S.A. & FLEMING, W.W. (1978). Sensitivities of the isolated ileal longitudinal smooth muscle-myenteric plexus and hypogastric nerve-vas deferens of the guinea-pig after chronic morphine pellet implantation. *J. Pharmac. exp. Ther.*, **204,** 54–66.

KLEE, W.A. & STREATY, R.A. (1974). Narcotic receptor sites in morphine-dependent rats. *Nature*, **248,** 61–63.

LEE, N.M., HO, I.K. & LOH, H.H. (1975). Effect of chronic morphine treatment on brain chromatin template activities in mice. *Biochem. Pharmac.*, **24,** 1983–1987.

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

MORITA, K. & NORTH, R.A. (1982). Opiate activation of potassium conductance in myenteric neurons: inhibition by calcium ions. *Brain Res.*, **242,** 145–150.

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. & VITEK, L.V. (1980). The effect of chronic morphine treatment on excitatory junction potentials in the mouse vas deferens. *Br. J. Pharmac.*, **68,** 399–405.

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.

SCHULZ, R. & CARTWRIGHT, C. (1974). Effect of morphine on serotonin release from myenteric plexus of the guinea-pig. *J. Pharmac. exp. Ther.*, **190,** 420–430.

SCHULZ, R. & CARTWRIGHT, C. (1976). Sensitization of the smooth muscle by prostaglandin E_1 contributes to reversal of drug-induced inhibition of the guinea-pig ileum. *Arch. Pharmac.*, **294,** 257–260.

SCHULZ, R. CARTWRIGHT, C. & GOLDSTEIN, A. (1974). Reversibility of morphine tolerance and dependence in guinea-pig brain and myenteric plexus. *Nature.*, **251,** 329–331.

SCHULZ, R., & GOLDSTEIN, A. (1973). Morphine tolerance and supersensitivity to 5-hydroxytryptamine in the myenteric plexus of the guinea-pig. *Nature*, **244,** 168–170.

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

TAKAGI, K., TAKAYANAGI, I., IRIKURA, T., NISHINO, K., ICHINOSEKI, N. & SHISHIDO, K. (1965). Responses of the isolated ileum of the morphine-tolerant guinea pig. *Arch. int. Pharmacodyn. Ther.*, **58,** 39–44.

WARD, A. & TAKEMORI, A.E. (1976). Studies on the narcotic receptor in the guinea-pig ileum. *J. Pharmac. exp. Ther.*, **199,** 117–123.

YAMAMOTO, H., HARRIS, R.A. LOH, H.H. & WAY, E.L. (1978). Effects of acute and chronic morphine treatments on calcium localization and binding in brain. *J. Pharmac. exp. Ther.*, **205,** 255–264.

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