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Effect of chronic morphine treatment on transmitter release from sympathetic varicosities of the mouse vas deferens

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1 Transmitter release from sympathetic varicosities of mouse vasa deferentia removed from animals which were chronically treated with morphine for 7 to 9 days has been evaluated.

2 In control preparations increasing the extracellular calcium concentration ($[Ca^{2+}]_o$) from 1 to 2 mM increased transmitter release by 3 fold while increasing $[Ca^{2+}]_o$ from 6 to 8 mM increased transmitter release by about 0.9 fold. Introduction of morphine (1.0 μM) produced a uniform decrease in transmitter release, shifting the relationship between transmitter release and $[Ca^{2+}]_o$ to the right.

3 Only sympathetic varicosities with probabilities of transmitter release greater than 0.01 were chosen for this study. In these varicosities the decrease in transmitter release induced by morphine in control preparations (bathed in $[Ca^{2+}]_o$ 2.0 mM) was not observed following 7 to 9 days of morphine treatment. When the morphine was acutely withdrawn from these preparations transmitter release was more than 6 times the average level of transmitter release from control preparations.

4 The morphine induced increase in facilitation of transmitter release while stimulating with short trains of nerve impulses was not observed when the preparations were removed from animals which had been exposed to morphine for 7 to 9 days. When these preparations were acutely withdrawn from morphine there was a further decrease in the level of facilitation and a significant increase in depression of transmitter release when compared to control.

5 The morphine induced decrease in probability of transmitter release when naive sympathetic varicosities *in vitro* were bathed with morphine (1 μM) was not observed following chronic morphine treatment of the animals for 7 to 9 days. When the morphine was acutely withdrawn from chronically morphine treated preparations the underlying increase in probabilities of transmitter release of sympathetic varicosities was unmasked.

Keywords: Morphine; tolerance; withdrawal; sympathetic; varicosities; vas deferens

Introduction

The morphine induced decrease in the amplitude of the excitatory junction potential (e.j.p.) is reduced when the extracellular calcium concentration is raised (Bennett & Lavidis, 1980; Illes *et al.*, 1980). Extracellular recordings of the nerve terminal impulse (NTI) and excitatory junction currents (e.j.cs) have demonstrated that morphine decreases transmitter release from sympathetic varicosities without affecting the propagation of the nerve impulse along terminal branches (Cunnane & Evans, 1988; Lavidis, 1995). This inhibitory affect of morphine on transmitter release can be reduced by procedures that are thought to increase the intracellular calcium concentration during nerve stimulation such as increasing the extracellular calcium concentration, during trains of nerve impulses and increasing the duration of the action potential with 4-aminopyridine (Lavidis, 1995). The results from the previous paper thus support the conjecture that the decrease in the probability of transmitter release induced by morphine is probably mediated either by a decrease in the amount of calcium ions entering the varicosities during nerve stimulation or by more rapid sequestration.

The morphine-induced decrease in e.j.p. amplitude does not occur if the preparation has been chronically morphine treated. This tolerance to morphine develops within 10 days of continuous morphine treatment (Einstein & Lavidis, 1984a) and is characterized by a shift of the relationship between e.j.p. amplitude and $[Ca^{2+}]_o$ back towards the naïve level. The increase in facilitation of the e.j.p. amplitudes in response to stimulation by trains of impulses seen in acute morphine treatment was reduced after chronic morphine treatment (Einstein & Lavidis, 1984a). These adaptive changes in transmitter release are evident when the morphine is acutely withdrawn and are characterized by a parallel shift of the relationship between e.j.p. amplitude and $[Ca^{2+}]_o$ to the left, a decrease in the level of facilitation as depression increased, in e.j.p. amplitude during stimulation by trains of impulses (Einstein & Lavidis, 1984b). These results suggest that the probability of transmitter release by sympathetic varicosities was increasing during chronic morphine treatment (Einstein & Lavidis, 1984b). This conjecture was investigated in the present study by recording transmitter release from single or small numbers of visualised sympathetic varicosities in the mouse isolated vas deferens.

Methods

Treatment of animals

Mice (Balb/c) aged between 4 and 5 weeks were treated with either saline or morphine for 7 to 9 days. Control animals received saline by sub-cutaneous injections (0.1 ml) three times per day. The dosage of morphine administered to animals was gradually increased using the following protocol: between day 1 and day 2 animals received 10 mg kg^{-1}; day 3 to day 4, 30 mg kg^{-1}; day 5 to day 9, 100 mg kg^{-1}. The volume of solution injected sub-cutaneously was kept constant at 0.1 ml. On the 8th or 9th day animals were injected with a dose of saline (control) or morphine at 5 h 30 min and then killed at 8 h 30 min. Both vasa deferentia were dissected from the animal and placed in Tyrode solution containing morphine (1 μM). This Tyrode solution was continuously gassed with 95°C O_2 and 5% CO_2. The temperature of this solution was kept between 16 and 18°C.

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Preparation of tissues

See the methods section of the previous paper (Lavidis, 1995). The preparation was continuously perfused at a rate of 3 ml per minute with a modified Tyrode solution of the following composition (mM): NaCl 123.4, KCl 4.7, $MgCl_2$ 1.0, NaH_2PO_4 1.3, $NaHCO_3$ 16.3, $CaCl_2$ 1.0–8.0, glucose 7.8 and morphine 0.001.

Stimulation

See the methods section of the previous paper (Lavidis, 1995).

Visualisation of the sympathetic varicosities

See the methods section of the previous paper (Lavidis, 1995).

Recording

See the methods section of the previous paper (Lavidis, 1995).

Data analysis

One hundred stimuli were recorded and collected on a Macintosh computer using Maclab as an A/D converter (rate of A/D conversion 3 kHz) and Scope software (version 3.3.4). The interval between two secretions following an impulse had to be greater than 0.4 ms or else they were measured as a single release. The frequency of this occurring was determined by comparing the largest e.j.cs with the largest amplitude of the spontaneous e.j.cs. Histograms of the number of releases, amplitude of evoked and spontaneous e.j.cs vs number of observations were constructed. The mean number of quanta released per impulse (quantal content, m_e) was determined either by dividing the total number of quanta released by the number of stimulations or by dividing the average amplitude of the e.j.cs by the average amplitude of the transmitter release e.j.cs.

Drugs

Drugs were dissolved in a second or third reservoir of about 200 ml capacity. Each reservoir was gassed with 95% O_2 and 5% CO_2. Solutions supplying the organ bath were changed by 3 way taps. Morphine hydrochloride and naloxone hydrochloride (gifts from the Department of Pharmacology, The University of Sydney) were dissolved in distilled H_2O and kept refrigerated as stock solutions at a concentration of 1 mM. Stock solutions of morphine (2 mg kg^{-1}, 6 mg kg^{-1} and 20 mg kg^{-1} in saline) were aliquoted under sterile conditions into vials and kept at 4°C. These were then used to treat animals chronically with morphine.

Results

The relationship between transmitter release and $[Ca^{2+}]_o$ in preparations following chronic morphine treatment of animals

The sympathetic varicosities on the surface of the mouse vas deferens were visible following $DiOC_2(5)$ treatment and fluorescence (Figure 1). There was no obvious visible difference between control (animals treated with saline) and chronically morphine treated (CMT) animals in the density of sympathetic varicosities on the muscle surface. Sympathetic varicosities with at least one e.j.c. recorded over the 100 consecutive stimulations at 0.1 Hz were chosen for study. When morphine was administered to control preparations there was a decrease in transmitter release without the nerve impulse being affected (Figure 2); this decrease in transmitter release did not occur if the preparation was isolated from animals which had received CMT (Figures 2 and 3). The decrease in transmitter release induced by morphine was inversely proportional to the extracellular calcium ion concentration ($[Ca^{2+}]_o$, Figure 3). There was a parallel shift to the right of control in the amplitude e.j.cs vs $[Ca^{2+}]_o$. In control preparations decreasing $[Ca^{2+}]_o$ from 8 to 1 mM significantly ($P<0.05$) increased the gradient of the relationship between e.j.c. amplitude and $[Ca^{2+}]_o$. Administration of morphine also significantly ($P<0.05$) increased the gradient of the relationship between e.j.c. amplitude and $[Ca^{2+}]_o$ having shifted the relationship to the right (Figure 3).

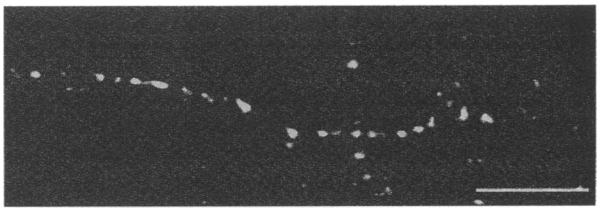

Figure 1 Sympathetic varicosities visualised by $DiOC_2(5)$-fluorescence. Only surface varicosities are clearly in focus and these were the brightest structures. The calibration bar is 20 μm.

The decrease in transmitter release induced by morphine did not occur when the preparations were obtained from animals that were CMT for 7 to 9 days (Figure 3). Preparations which were obtained from CMT animals had significantly ($P<0.05$) greater probabilities of transmitter release indicated by a decrease in the number of failures to release transmitter during nerve stimulation and greater e.j.c. amplitudes when compared to controls (Figure 3). When $[Ca^{2+}]_o$ was increased from 2 to 4 mM the slope of the relationship between transmitter release and $[Ca^{2+}]_o$ was: control, 1.45 ± 0.41 (mean$\pm$s.d.); control with morphine, 2.0 ± 0.9; CMT, 1.1 ± 0.5; CMT and without morphine, 0.7 ± 0.3 ($n\geqslant7$). There was a significant ($P<0.05$) increase in the amount of transmitter release and a decrease in the slope of the relationship between transmitter release and $[Ca^{2+}]_o$ between control preparations with morphine and CMT preparations with morphine, and between control preparations and CMT preparations which had morphine acutely withdrawn. Acute withdrawal from morphine of CMT preparations significantly increased transmitter release from sympathetic varicosities vs CMT with morphine, these preparations had maximum levels of transmitter release that would be expected from preparations bathed in 5 mM $[Ca^{2+}]_o$ rather than 2 mM (Figure 3).

Facilitation in transmitter release during trains of nerve impulses in preparations chronically morphine treated

The level of facilitation in transmitter release due to a 0.5 Hz train of 5 impulses was determined for varicosities which had probabilities of transmitter release higher than 0.01. In control preparations there was no increase in the average e.j.c. amplitude of the 2nd to 5th impulses instead a decrease was observed that may be due to autoinhibition and possibly depletion (Figure 4). This decrease could be converted to facilitation when the α_2-adrenoceptor antagonist (yohimbine or idazoxane) was used to inhibit the α_2-adrenoceptors (located on the presynaptic membrane) without affecting e.j.c. amplitude of the 1st impulse (Lavidis, unpublished observation). Acute morphine (1.0 μM) decreased the average amplitude of the 1st e.j.c. in a train and increased the level of facilitation in transmitter release produced by the 2nd to 5th impulses (Figure 4). Stimulation with short high frequency trains of nerve impulses partially reversed the morphine-induced decrease in e.j.c. amplitude of the 1st impulse. Transmitter release from varicosities of CMT animals was significantly greater when compared to controls and the level of facilitation in transmitter release was as a consequence reduced (Figure 4). The level of facilitation in CMT preparations when bathed in morphine containing solutions was similar to the level of facilitation shown by control preparations. When the morphine was removed from these varicosities there was an increase in the

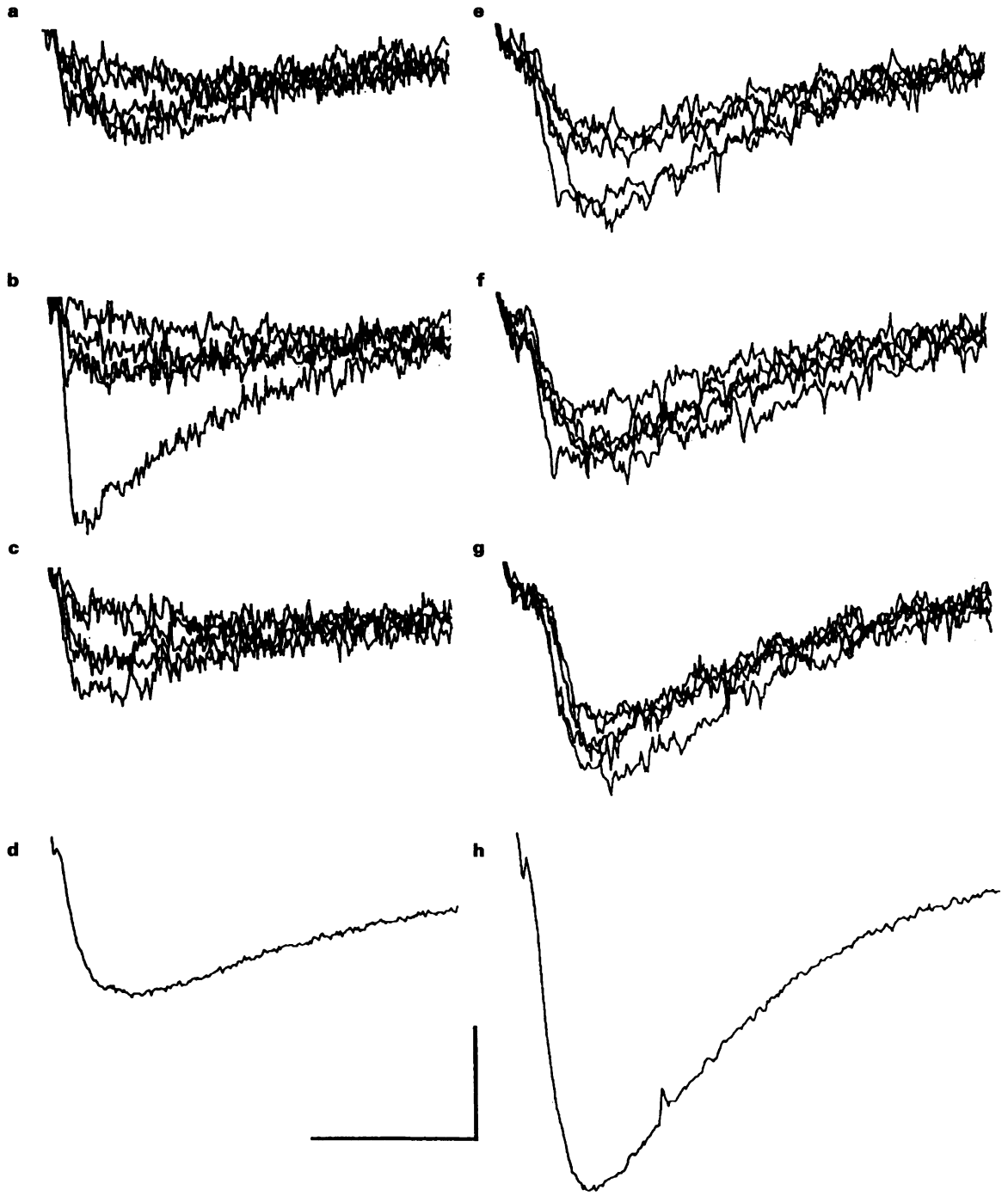

Figure 2 Examples of excitatory junction currents (e.j.cs) recorded from a preparation which had been chronically treated with saline (a,b,c,d) and a preparation which had been chronically morphine treated for 9 days (e, f, g, h). In (e) to (h) the morphine was withdrawn from the bath 15 min before these recordings were taken. The $[Ca^{2+}]_o$ was 2 mM. In (a b c e f and g) five consecutive recordings of e.j.cs are shown for each trace. In (d) and (h) the average of 100 traces is shown. Calibration bars indicate: for (a–c) and (e–g), 15 μV and 20 ms; (d) and (h), 5 μV and 20 ms.

amplitude of e.j.cs of the 1st impulse and a greater depression of the 2nd to 5th impulse when compared to control preparations (Figure 4).

The probability of transmitter release from sympathetic varicosities chronically morphine treated

In control preparations when $[Ca^{2+}]_o$ was 2 mM the majority ($n = 103$) of varicosities studied (63%) showed no evoked transmitter release over the 100 stimulations recorded even though the nerve impulse and s.e.j.cs were recorded. The remaining 37% had probabilities of transmitter release which ranged from 0.01 to 0.44 in $[Ca^{2+}]_o$ of 2 mM (Figure 5a). Most of the 37% of varicosities which showed release over the stimulus period had transmitter release probabilities of less than 0.15. Morphine (1.0 μM) decreased the probability of transmitter release of every varicosity studied without affecting the regularity and amplitude of the nerve terminal impulse. Following morphine administration the highest probability of transmitter release was 0.1 with most falling below 0.05 (Figure 5b)

The probability of transmitter release of varicosities which had been continuously exposed to morphine was higher than for control preparations when both were exposed to morphine (compare the open columns in Figure 5a with Figure 5b). In CMT preparations approximately 53% of the varicosities

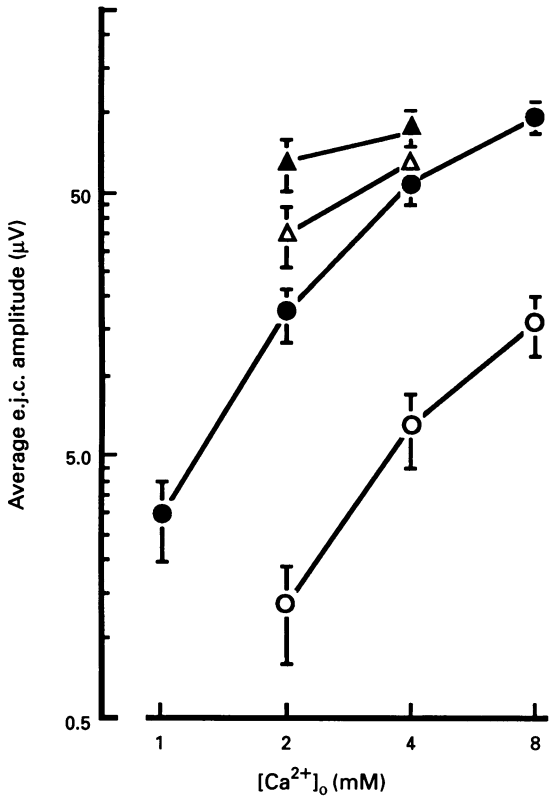

Figure 3 The effect of $[Ca^{2+}]_o$ on average excitatory junction current (e.j.c.) amplitude. (●) Control; (○) control with acute morphine (1.0 μM); (△) chronically morphine treated with morphine (1.0 μM); (▲) chronically morphine treated without morphine in the bath (withdrawal). The size of the electrode used was 10 μm and the number of varicosities recorded from was kept constant at 3. The average amplitude ± s.e.mean is shown for at least 7 preparations.

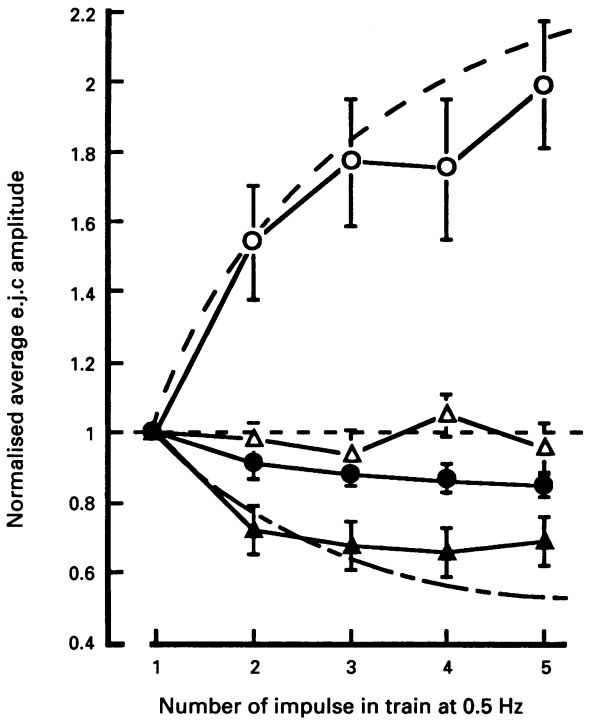

Figure 4 Effect of stimulating sympathetic varicosities with short trains of five impulses from control and chronically morphine treated (CMT) preparations. (●) Control; (○) control with morphine (1.0 μM); (△) CMT with morphine (1.0 μM); (▲) CMT without morphine (withdrawal). The size of the electrode used was 10 μm and the number of varicosities recorded from was kept constant at 3. The frequency of stimulation used was 0.5 Hz. The $[Ca^{2+}]_o$ bathing the preparations was 2 mM. The average level of facilitation ± s.e.mean is shown ($n > 7$). Points above the short dashed line indicate facilitation while points below this line indicate depression. Long dashed lines indicate facilitation without any depression. Short and long dashed lines indicate depression determined by using a time constant of depression of 4s.

studied ($n = 107$) had probabilities of transmitter release less than 0.01 even though the nerve impulse and s.e.j.cs were always recorded. The remaining 47% of sympathetic varicosities had probabilities of transmitter release ranging from 0.01 to 0.7 (Figure 5b, open columns). When the morphine was withdrawn from these CMT sympathetic varicosities there was a significant ($P < 0.05$) increase in the probability of transmitter release of varicosities (0.01–0.7 increased to 0.25–>0.8) so that release from these varicosities was equivalent to the level of release expected from control preparations bathed in 4 to 5 mM $[Ca^{2+}]_o$ (Figure 3). Only 16% of the varicosities studied showed no evoked transmitter release during 100 nerve stimulations while the remaining 84% had probabilities which ranged from 0.01 to >0.8 (Figure 5b, hatched and solid columns). In situations where the probability of transmitter release was very high it was difficult to estimate accurately the probability of transmitter release since complex positive and negative potentials were continually recorded. This was very common when the $[Ca^{2+}]_o$ was >6.0 mM or when the preparation was extracted from animals which had been chronically morphine treated.

Discussion

The effects of chronically treating sympathetic varicosities with morphine

Lineweaver-Burk plot of e.j.p. vs $[Ca^{2+}]_o$ demonstrated that CMT had induced a decrease in the $K[Ca^{2+}]_o$ with no change in the maximum e.j.p. amplitude (y-intercept of the linear regression lines) prompting Einstein & Lavidis (1984b) to suggest that an increase in the probability of transmitter release by sympathetic varicosities was occurring during CMT (Einstein & Lavidis, 1984b). Exploiting recent advances in our ability to record the NTI and e.j.cs (Brock & Cunnane, 1987; 1988) from visualised sympathetic varicosities using small diameter extracellular electrodes (Lavidis & Bennett, 1992; 1993a,b), the probability of transmitter release from sympathetic varicosities of mouse vasa deferentia extracted from animals which were treated with morphine for 7 to 9 days has been evaluated.

The probability of transmitter release from sympathetic varicosities is dependent on the $[Ca^{2+}]_o$, the frequency of nerve stimulation and the type of tissue studied. In the mouse vas deferens, the probability of transmitter release when the extracellular calcium concentration was 2 mM varied considerably. The probability of transmitter release was less than 0.01 for 63% of the sympathetic varicosities studied and >0.01 to <0.45 for the remaining 37% ($n = 104$). When $[Ca^{2+}]_o$ was increased to 4 mM the probability of transmitter release was increased so that 45% of sympathetic varicosities studied had probabilities of transmitter release <0.01 and >0.01 to <0.7 for the remaining 55% ($n = 107$). In this study sympathetic varicosities with probabilities of transmitter release greater than 0.01 were only studied. In CMT preparations finding sympathetic varicosities with probabilities of transmitter release >0.01 was relatively easy ie. 84% of the varicosities studied in CMT preparations had probabilities of transmitter release >0.01 compared to 37% for controls. Also the highest probability of transmitter release recorded in CMT preparations was >0.7 while for controls was 0.44. The accuracy of estimating the probability of transmitter release from release sites is dependent on the number of varicosities recorded from and the extent of variation in the probability of transmitter

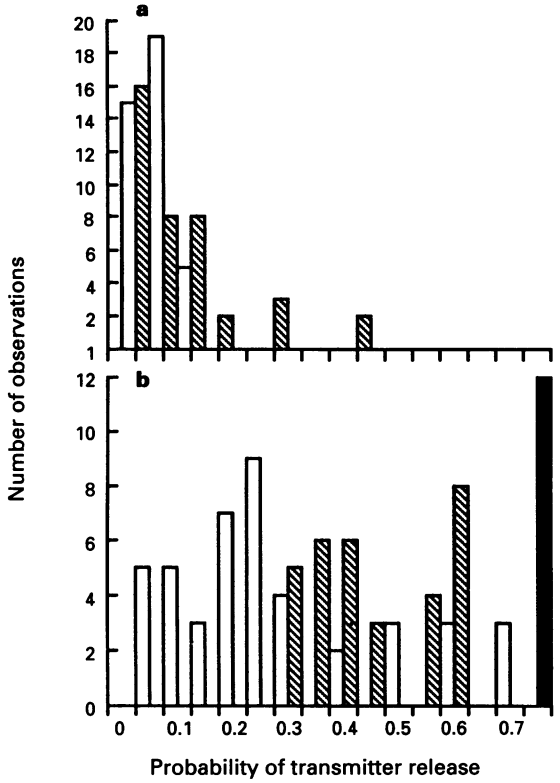

Figure 5 Probabilities of transmitter release from sympathetic varicosities bathed in 2 mM $[Ca^{2+}]_o$. (a) Control preparations; (b) chronically morphine treated preparations. Hatched columns indicate no morphine present in the bathing solution; open columns indicate the presence of morphine (1 μM) in the bathing solution. The solid column in (b) indicates varicosities with probabilities of transmitter release greater than 0.8, at these high levels of transmitter release complex signals were continually recorded making estimates of transmitter release probability very difficult.

release from the recorded varicosities. The ideal situation would be to record from only single sympathetic varicosities and this was attempted in the present study. However, given the complex three-dimensional array of sympathetic varicosities throughout the smooth muscle syncytium, accurate estimates of the variability in the probability between sympathetic varicosities could not easily be made, instead the relative differences in probability of transmitter release between control and CMT sympathetic varicosities has been made. This study has shown that the average probability of transmitter release from sympathetic varicosities increased during CMT. What then are the physical changes responsible for the increase in probability of transmitter release from sympathetic varicosities of animals which had been CMT?

Effect of chronic morphine treatment on calcium ion influx in varicosities during nerve stimulation

In untreated varicosities, increasing $[Ca^{2+}]_o$ from 1 to 2 mM increased transmitter release by 3 fold while increasing $[Ca^{2+}]_o$ from 6 to 8 mM increased transmitter release by about 0.9 fold. Bath application of morphine (1.0 μM) produced a uniform decrease in transmitter release, shifting the e.j.c. amplitude vs $[Ca^{2+}]_o$ relationship to the right. The decrease in transmitter release induced by morphine in control preparations (bathed in $[Ca^{2+}]_o$ 2.0 mM) was not observed following 7 to 9 days of morphine treatment. When morphine was acutely withdrawn from these CMT preparations, transmitter release was more than 6 times the average level of transmitter secretion of control preparations. The increase in transmitter release following CMT may occur as a result of an increase in the density of calcium channels around the release zone, or an increase in the duration of calcium channel opening during nerve stimulation leading to a higher intracellular calcium concentration, or due to a decreased sequestration of intravaricosity calcium on a pulse to pulse basis.

Changes in the rate of calcium ion sequestration from within the sympathetic varicosities

Calcium ions which have entered the varicosities during nerve stimulation are quickly sequestered (McGraw *et al.*, 1980; Zucker, 1985). If the terminal is stimulated using trains of nerve impulses there is an increase in the amount of transmitter release (Burnstock *et al.*, 1964; Bennett & Lavidis, 1980; Einstein & Lavidis, 1984a,b). This may arise as a consequence of an increased intravaricosity calcium ion concentration produced by residual calcium ions and/or extracellular calcium ions entering the terminal during nerve stimulation inducing the release of intra terminal stores of calcium (Smith & Cunnane, 1994). Morphine (1 μM) decreased the e.j.c. amplitude of the 1st impulse in a train but failed to suppress the amplitude of e.j.cs by subsequent impulses resulting eventually in a reversal of the initial effect of morphine. This morphine-induced increase in facilitation of transmitter release produced by short trains of impulses was not observed when preparations were dissected from animals which had been CMT. A possible reason for this lack of facilitation of transmitter release in CMT preparations is the large increase in probability of transmitter release of sympathetic varicosities leaving very few low probability release sites remaining to be recruited. However, when considering evoked transmitter release during trains of impulses it is very difficult to isolate the effects of presynaptic inhibition and depletion of available (docked) vesicles from facilitation. This is specially relevant when the level of transmitter release has been increased.

Changes in the rate of vesicle delivery to the release area

The probability of transmitter release is presumably determined initially by at least the level of free intracellular calcium ions and the availability of vesicles for transmitter release at the release site. During a high frequency train of impulses there is first an increase in the probability of transmitter release (facilitation) followed by a decrease (depression). Two possible mechanisms are responsible for this depression; first, the existence of presynaptic adrenoceptors (α_2-adrenoceptors) which are activated by previously secreted noradrenaline inducing an autoinhibition (Msghina & Stjarne, 1993) which lasts for about 10 s (time constant = 4 s, Lavidis, unpublished observation). Secondly, even after blocking the α_2-adrenoceptors with high concentrations of yohimbine there is a decrease in the e.j.p. amplitude during long trains of impulses (Lavidis, unpublished observation). It is possible that CMT causes an increase in the mobilization of vesicles. Some evidence exists for this idea from the observation that synapsin 1 (which is important in vesicle transport) is up-regulated in a dose-dependent manner by chronic administration of opiates (Nah *et al.*, 1993). The increase in synapsin 1 levels following chronic opiate treatment was blocked by an opiate antagonist (Nah *et al.*, 1993).

This study has demonstrated that chronic morphine treatment induces an increase in the probability of transmitter release of sympathetic varicosities. This effect is possible since a large number of sympathetic varicosities are normally relatively silent and can be recruited during prolonged presynaptic inhibition.

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References

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. Pharmacol.*, **69**, 185–191.

BROCK, J.A. & CUNNANE, T.C. (1987). Relationship between the nerve action potential and transmitter release from sympathetic postganglionic nerve terminals. *Nature*, **326**, 605–607.

BROCK, J.A. & CUNNANE, T.C. (1988). Electrical activity at the sympathetic neuroeffector junction in the guinea-pig vas deferens. *J. Physiol.*, **399**, 607–632.

BURNSTOCK, G., HOLMAN, M.E. & KURIYAMA, H. (1964). Facilitation of transmission from autonomic nerve to smooth muscle of guinea-pig vas deferens. *J. Physiol.*, **172**, 31–49.

CUNNANE, T.C. & EVANS, R.J. (1988). Effects of morphine on electric activity in sympathetic nerve terminals of the mouse vas deferens. *Proc. Br. J. Pharmacol.*, **95**, 544P.

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

EINSTEIN, R. & LAVIDIS, N.A. (1984b). The dependence of excitatory junction potential amplitude on the external calcium concentration in mouse vas deferens during narcotic withdrawal. *Br. J. Pharmacol.*, **83**, 863–870.

ILLES, P., ZIEGLOGANSBERGER, W. & HERZ, A. (1980). Calcium reverses the inhibitory action of morphine on neuroeffector transmission in the mouse vas deferens. *Brain Res.*, **191**, 511–522.

LAVIDIS, N.A. (1995). Effect of morphine on the nerve terminal impulse and transmitter secretion from sympathetic varicosities innervating the mouse vas deferens. *Br. J. Pharmacol.*, **116**, 2852–2859.

LAVIDIS, N.A. & BENNETT, M.R. (1992). Probabilistic secretion of quanta from visualised sympathetic nerve varicosities in mouse vas deferens. *J. Physiol.*, **454**, 9–26.

LAVIDIS, N.A. & BENNETT, M.R. (1993a). Probabilistic secretion of quanta from successive sets of visualised varicosities along single sympathetic nerve terminals. *J. Auton. Nerv. Syst.*, **43**, 41–50.

LAVIDIS, N.A. & BENNETT, M.R. (1993b). Sympathetic innervation of the surface of the mouse vas deferens. *J. Auton. Nerv. Syst.*, **45**, 87–100.

MCGRAW, C.F., SOMLYO, A.V. & BLAUSTEIN, M.P. (1980). Localisation of calcium in presynaptic nerve terminals. An ultrastructural and electron microscope analysis. *J. Cell. Biol.*, **85**, 228–241.

MSGHINA, M. & STJARNE, L. (1993). Sympathetic transmitter release in rat tail artery and mouse vas deferens: facilitation and depression during high frequency stimulation. *Neurosci. Letts.*, **153**, 17–41.

NAH, S.Y., SAYA, D. & VOGEL, Z. (1993). Long-term opiate exposure leads to increase in synapsin I in rat spinal cord-dorsal root ganglion cocultures. *J. Neurochem.*, **60**, 1147–1150.

SMITH, A.B. & CUNNANE, T. (1994). Ryanodine-sensitive neurotransmitter release in the guinea-pig isolated vas deferens. *J. Physiol.*, **481**, 27P.

ZUCKER, R.S. (1985). Calcium diffusion models and transmitter release in neurones. *Fed. Proc.*, **44**, 2950–2952.

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