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Effect of chronic morphine treatment on α_2-adrenoceptor mediated autoinhibition of transmitter release from sympathetic varicosities of the mouse vas deferens

[1]Shanker Karunanithi & *,[2]Nickolas A. Lavidis

[1]Department of Physiology, University of Toronto, Toronto, Ontario, M5S 1A8, Canada and [2]Department of Physiology and Pharmacology, The University of Queensland, Queensland, Australia, 4072

1 The effect of chronic morphine treatment (CMT) on sympathetic innervation of the mouse vas deferens and on α_2-adrenoceptor mediated autoinhibition has been examined using intracellular recording of excitatory junction potentials (EJPs) and histochemistry.

2 In chronically saline treated (CST) preparations, morphine (1 μM) and the α_2-adrenoceptor agonist (clonidine, 1 μM) decreased the mean amplitude of EJPs evoked with 0.03 Hz stimulation by $81 \pm 8\%$ ($n=16$) and $92 \pm 6\%$ ($n=7$) respectively. In CMT preparations, morphine (1 μM) and clonidine (1 μM) decreased mean EJP amplitude by $68 \pm 8\%$ ($n=7$) and $79 \pm 8\%$ ($n=7$) respectively.

3 When stimulating the sympathetic axons at 0.03 Hz, the mean EJP amplitude recorded from smooth muscles acutely withdrawn from CMT was four times greater than for CST smooth muscles (40.7 ± 3.8 mV, $n=7$ compared with 9.9 ± 0.3 mV, $n=7$).

4 Part of the increase in mean EJP amplitude following CMT was produced by a 31% increase in the density of sympathetic axons and varicosities innervating the smooth muscle.

5 Results from the present study indicate that the effectiveness of α_2-adrenoceptor mediated autoinhibition is only slightly reduced in CMT preparations. Most of the cross tolerance which develops between morphine, clonidine and α_2-adrenoceptor mediated autoinhibition occurs as a consequence of increased efficacy of neuromuscular transmission which is produced by an increase in the probability of transmitter release and an increase in the density of sympathetic innervation.
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Abbreviations: $[Ca^{2+}]_o$, extracellular calcium concentration; CMT, chronic morphine treatment; CST, chronic saline treatment; EJP, excitatory junction potential

Introduction

Activation of presynaptically located opioid receptors with morphine or α_2-adrenoceptors with noradrenaline or clonidine results in inhibition of transmitter release (Henderson *et al*., 1972; Starke, 1972; see reviews by Starke, 1977; 1987; Illes & Starke, 1983; Starke *et al*., 1989). Activation of α_2-adrenoceptors is thought to autoinhibit transmitter release during stimulation of the sympathetic nerves with trains of impulses since α_2-adrenoceptor antagonists greatly reduce depression (Ramme *et al*., 1986; Brock *et al*., 1990). An interaction between α_2-adrenoceptors and opioid receptors has been demonstrated only during stimulation by trains of nerve impulses (Ramme *et al*., 1986) and not during single impulse nerve stimulation (Limberger *et al*., 1988a). The level of autoinhibition is thought to increase as the concentration of noradrenaline increases in the extrasynaptic space during stimulation of the nerves with trains of impulses (Brock *et al*., 1990).

Although opiates and α_2-adrenoceptor agonists act on different presynaptic receptors, they seem to operate through a common mechanism which results in inhibition of transmitter release. It has been suggested that there is a direct interaction between the receptors rather than sharing of a common transduction system (Illes & Noremberg, 1990). However an action further down stream from the receptor level is indicated by the observation that the inhibitory effects of opiate and α_2-adrenoceptor agonists can be overcome by procedures which are thought to increase intracellular levels of free calcium (Bennett & Lavidis, 1980; Illes *et al*., 1980; Alberts *et al*., 1981; Lavidis, 1995a). More recently clonidine (1 μM) was shown to decrease the intravaricosity calcium concentration during trains of nerve stimulation by 45% (Brain & Bennett, 1997). In preliminary studies morphine (1 μM) was also shown to decrease intravaricosity calcium concentration following single nerve stimulation by about 17–25% (Lavidis, unpublished observation).

Tolerance to the effects of morphine on transmitter release is observed following CMT. This adaptive change becomes evident when the morphine is acutely withdrawn and is observed as an increase in transmitter release (Montel *et al*., 1975; Brodie *et al*., 1980; Einstein & Lavidis, 1984a,b, Lavidis, 1995b). Although clonidine has been used to alleviate some of the symptoms of opiate withdrawal, a simple explanation of the interaction between opioid-receptors and α_2-adrenoceptors which can account for all

*Author for correspondence; E-mail: lavidis@plpk.uq.edu.au

previous observations has not materialized. α_2-adrenoceptor agonists: (a) are used as an alternative to methadone to reduce opiate withdrawal symptoms (Gold *et al*., 1978), (b) prevent the contracture induced by naloxone of tolerant guinea-pig ileum (Alfaro *et al*., 1990) and (c) are effective only when the morphine has been removed from the bathing solution (Gillan *et al*., 1979). α_2-adrenoceptor antagonists: (a) potentiate the effect of morphine withdrawal (Dwoskin *et al*., 1983) and (b) delay the development of morphine tolerance (Kihara & Kaneto, 1986; Alguacil *et al*., 1987). In addition, cross-tolerance between opiates and α_2-adrenoceptor agonists has been demonstrated in a number of other studies (Yamazaki & Kaneto, 1985; Solomon & Gebhart, 1988; Stevens *et al*., 1988). Overall these observations are inconclusive in identifying the level at which morphine and clonidine interact. In the present inquiry we have used CMT mouse vasa deferentia to examine the effectiveness of α_2-adrenoceptor mediated autoinhibition following enhancement of transmitter release from sympathetic varicosities by acute morphine withdrawal.

Methods

Treatment of animals

Male mice (Balb/c) aged between 5 and 6 weeks postnatal were treated with either saline or morphine for 7–9 days. Chronically saline treated (CST) animals received saline by subcutaneous injections (0.1 ml) three times per day. Morphine was administered to CMT animals according to the following protocol which was approved by the University of Queensland Animal Experimentation Ethical Committee (AEEC approval number: PHYS/PH/086/97): between days 1 and 2 animals received 10 mg kg^{-1}; days 3 and 4, 30 mg kg^{-1}; days 5 to 9, 100 mg kg^{-1}. By following this protocol all the animals survived the treatment. We gradually increased the dosage of morphine administered to these animals to a dose well above the normal therapeutic dose administered to humans. Since we know from previous studies (Einstein & Lavidis, 1984b) that tolerance to morphine can easily be induced following this protocol. The volume of solution injected subcutaneously was kept constant at 0.1 ml. Thus the level of morphine was gradually increased over the treatment period. After 8 or 9 days of treatment animals were injected with saline (CST animals) or a dose of morphine (CMT animals) and then killed by cervical fracture 3 h later. Both vasa deferentia were dissected from each animal and placed in Tyrode's solution containing morphine (1 μM). After 2 h the morphine was acutely withdrawn from the preparations.

Preparation of tissues

Both vasa deferentia were dissected free from the surrounding tissues and used for either electrophysiological or morphological analysis. One vas deferens was used to measure the density of varicosities and axons using fluorescence confocal microscopy. The other vas deferens was pinned to the Sylgard covered base of a 3 ml capacity organ bath and examined electrophysiologically. The organ bath was continually perfused at the rate of 3 ml per min with modified Tyrode's 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; $CaCl_2$ 2.0; glucose 7.8, and the temperature maintained between 32–34°C. The reservoir supplying the bath was gassed with 95% O_2 and 5% CO_2. The pH was maintained at 7.3.

Electrical stimulation

The prostatic end of the vas deferens was gently drawn into a pipette filled with modified Tyrode's solution. Silver/silver chloride wires, one within the pipette and the other on the outside, were used to stimulate the axons innervating the mouse vas deferens using square wave pulses (0.1 ms duration and 15–30 V amplitude). A Grass Instruments stimulator (SD40) with a stimulus isolator (SIU5) was used to produce trains of square wave pulses.

Recording

Conventional intracellular recording techniques were used to monitor changes in membrane potential. Microelectrodes filled with 2 M KCl and having resistances of $\geqslant$50 MΩ were used to record the resting membrane potential of smooth muscle cells and EJPs. The distance of the intracellular recording electrode from the stimulating electrode was approximately 1.2 mm. Recordings of EJPs were accepted for analysis if the resting membrane potential was < -50 mV and did not vary by more than 10% over the recording period. If this criteria was met, recordings of EJPs were taken in the presence and absence of morphine (1 μM) or clonidine (1 μM). EJPs were evoked by trains of stimuli at 0.03 Hz or by trains of five stimuli at 1.0 Hz delivered every 50 s. EJPs were digitized using MacLab software (Scope, version 3.3.4; ADI Instruments, Australia) and stored on a Macintosh (DuoDock) computer for subsequent analysis.

Data analysis

The average EJP amplitude during trains of stimuli at 0.03 Hz was determined by averaging 100 consecutive EJPs. For each recording site, the average EJP amplitude was determined. The mean EJP amplitude of a number of intracellular recordings were averaged and the standard error of these determined to make comparisons between different treatments. The means were compared using Student's *t*-test; $P<0.05$ was taken to be statistically significant.

For EJPs recorded during trains of 1 Hz stimulation, the 2nd to 5th EJP amplitudes in each train were normalized with respect to the 1st EJP in the train. The means$\pm$s.e.mean from each set of normalized values were determined.

Visualization of sympathetic varicosities and axons

Isolated vasa deferentia were placed in Tyrode's solution containing 3,3′-diethyloxardicarbocyanine iodide ($DiOC_2(5)$, 0.1 μM) for 30 s and then washed with Tyrode's solution for 3 min in minimal illumination. The whole vas deferens was then placed on a glass slide and a cover slip placed on top. The preparation was examined using a confocal microscope (BioRad MRC600) with a 63 times objective. A z-series of each field was recorded up to a depth of 15 μm at 1 μm steps.

Images were collapsed into a single frame and all the sympathetic varicosities and axons counted using NIH image analysis. The specificity of $DiOC_2(5)$-fluorescence to label only sympathetic varicosities was demonstrated by Lavidis and Bennett (1993).

Drugs

Drugs were dissolved in a second or third reservoir of 200 ml capacity. Each reservoir was gassed with 95% O_2 and 5% CO_2. Solutions were directed to the preparation by means of 3-way taps. Morphine hydrochloride, clonidine hydrochloride and idazoxane hydrochloride (gifts from the Department of Pharmacology, University of Sydney) were dissolved in distilled H_2O and stored at 4°C as 1 mM stock solutions. Stock solutions of morphine (2 mg ml^{-1}, 6 mg ml^{-1} and 20 mg ml^{-1} in saline) were aliquoted into vials using aseptic procedures and kept at 4°C. The contents of these vials were used to chronically treat animals with morphine.

Results

Effect of CMT on density of sympathetic varicosities innervating the smooth muscle of vasa deferentia

We examined the density of $DiOC_2(5)$-fluorescent structures in a volume of smooth muscle measuring 45 μm × 45 μm × 15 μm. X – Y scans were acquired at various depths from the surface of the tissue to a maximum depth of 15 μm using a confocal microscope and optical sectioning. All the images were then compressed to a single frame measuring 45 μm × 45 μm and used to count the number of fluorescent structures. For CST preparations, 68.5 ± 4.2 (mean ± s.e.mean, $n = 91$) $DiOC_2(5)$-fluorescent structures were counted per 30,375 μm^3 and for CMT preparations 89.8 ± 5.3 ($n = 87$) $DiOC_2(5)$-fluorescent structures per 30,375 μm^3. There was a significant ($P < 0.001$) increase (31%) in the density of $DiOC_2(5)$-fluorescent structures in CMT preparations.

Effect of acute morphine or clonidine administration on EJP amplitude in CST and CMT preparations

In CST preparations, morphine (1 μM) and clonidine (1 μM) decreased the mean amplitude of EJPs evoked by trains of stimuli at 0.03 Hz (Figure 1). When preparations were stimulated at 30 V intensity and 0.1 ms duration at 0.03 Hz, the average EJP amplitude was 9.9 ± 0.3 mV ($n = 7$) for CST preparations and 40.7 ± 3.8 mV ($n = 7$) for CMT preparations (Figure 1). In CST preparations, morphine (1 μM) induced an 81 ± 8% ($n = 16$) decrease in EJP amplitude while in separate CST preparations clonidine induced a 92 ± 6 % ($n = 7$) decrease. In CMT preparations, morphine (1 μM) produced a 68 ± 8% ($n = 7$) decrease in EJP amplitude. In separate CMT preparations, clonidine (1 μM) produced a 79 ± 8% ($n = 7$) decrease in EJP amplitude (Figure 1). If we compare the mean EJP amplitudes (Figure 1) of CST preparations without morphine or clonidine with CMT preparations in the presence of morphine (1 μM) or clonidine (1 μM) no significant difference is observed. This apparent tolerance to both morphine and clonidine reflects an underlying significant increase in transmitter release from sympathetic varicosities that had received CMT.

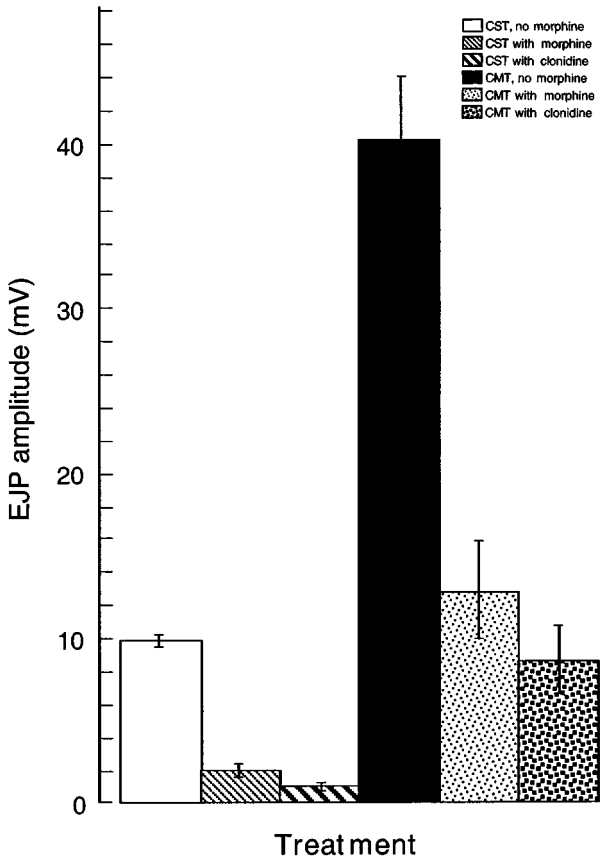

Figure 1 Effect of morphine and clonidine on EJP amplitude in CST and CMT preparations. The mean EJP amplitude ± s.e.mean ($n \geqslant 7$) is shown on the ordinate and treatment of the vasa deferentia is indicated by the key. The concentration of morphine and clonidine added to the bathing solution was 1 μM. Nerves were stimulated by trains of impulses, 30 V (strength), 0.1 ms (duration) and 0.03 Hz.

Effect of stimulus intensity on the amplitude of EJPs in CST and CMT preparations

Throughout these experiments the distance between the stimulating and recording electrode was 1.2 mm. Increasing the stimulus intensity from 15 to 30 V did not significantly change the amplitude of the EJPs in CST preparations (Figure 2, filled triangles). This result indicated that pulses of 15 V and 0.1 ms duration achieved supermaximal stimulation. There was no significant difference between the EJP amplitudes of CST preparations before and after administration of 1 μM idazoxane (Figure 2, filled circles). Morphine (1 μM) significantly ($P < 0.05$) decreased the amplitude of EJPs at stimulus intensities between 15 and 30 V. Increasing the stimulus intensity did not reduce the morphine induced decrease in EJP amplitude (Figure 2, filled squares).

In CMT preparations, increasing the stimulus intensity from 15 to 30 V produced an increase in mean EJP amplitude (Figure 2, open triangles). Administration of morphine (1 μM) to the bathing solution produced a proportional decrease in EJP amplitude (Figure 2, open squares). The

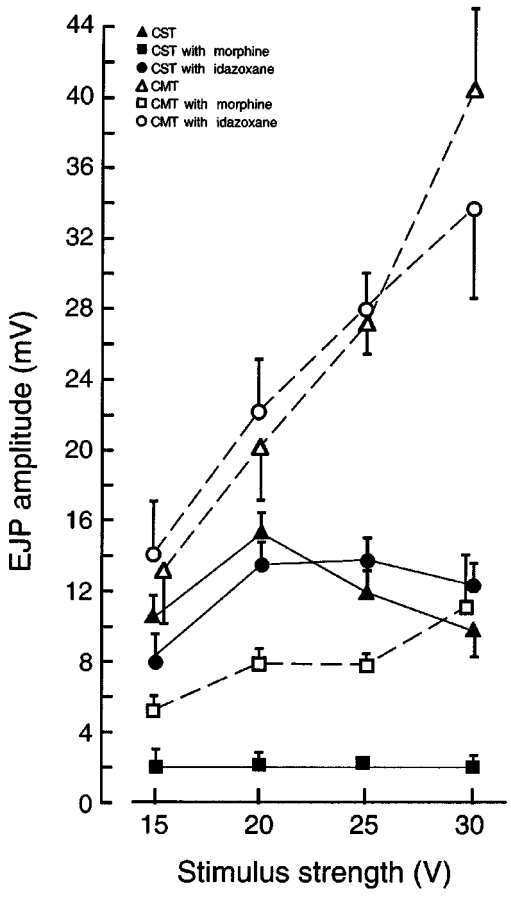

Figure 2 Effect of increasing the stimulus intensity on the mean amplitude of EJPs from CST and CMT preparations. Symbols represent different treatment groups as indicated by the key. Symbols indicate the mean EJP amplitude ± s.e.mean ($n \geqslant 7$) in mV. The nerves were stimulated at 0.03 Hz.

mean amplitude of EJPs recorded from CMT preparations when morphine was present in the bathing solution was significantly greater ($P<0.05$) at all stimulus intensities examined compared with EJP amplitudes recorded from CST preparations bathed in morphine (1 μM). Addition of idazoxane (1 μM) to CMT preparations did not alter the amplitude of EJPs (Figure 2, open triangles versus open circles).

α_2-adrenoceptor mediated autoinhibition of transmitter release from CST and CMT preparations

We next examined the effect of CMT on α_2-adrenoceptor mediated autoinhibition by examining EJP amplitude during frequent (1 Hz) stimulation. During such stimulation at least two mechanisms are thought to operate simultaneously, changing the amplitude of EJPs. Facilitation/augmentation which are thought to result from residual calcium and a concurrent depression mediated partly by autoinhibition. In the present study, we aimed to investigate the effects of CMT on autoinhibition, which however, necessitated examining also facilitation/augmentation. In CST preparations, stimulation of the vas deferens with trains of 5 impulses at 1 Hz resulted in an increase of the EJP amplitude from stimulus to stimulus (facilitation/augmentation). By the 5th stimulus, the EJP was about 1.4 times greater in amplitude than the EJP evoked by the first stimulus in a train (Figure 3A). The level of facilitation/augmentation did not vary significantly when the stimulus intensity was increased from 15 V to 30 V (Figure 3A).

The predicted level of facilitation/augmentation from impulse to impulse was calculated using a decay time constant for facilitation of 6 s and the value of facilitation at the 2nd nerve impulse (when autoinhibition is thought to be minimal) in control preparations (Figures 3 and 4; dashed lines, for a more detailed account of the calculation see Bennett & Florin, 1975). At all stimulus intensities examined, the experimental level of facilitation/augmentation was always less than the predicted level of facilitation/augmentation (Figure 3A). For example, the predicted and experimental level of facilitation/augmentation for the 5th nerve impulse was 1.9 and 1.4 respectively. The difference between the predicted and experimental values were highly significant ($P<0.01$) for the 4th and 5th responses (Figure 3A). When idazoxane (1 μM) was added to the bathing solution, there was an increase in the level of facilitation/augmentation such that no significant difference between the predicted and experimental levels of facilitation/augmentation existed at any stimulus intensity examined (Figure 3C). These results indicate that the discrepancy between the experimental and predicted levels of facilitation/augmentation is due to α_2-adrenoceptor mediated autoinhibition (in Figures 4A,B, compare filled circles with open circles and Figure 4C compare the difference between the dashed line and light shaded bars).

In acutely withdrawn preparations from CMT, there was an inverse relationship between the intensity of nerve stimulation and the level of facilitation/augmentation in EJP amplitude (Figure 3B). At a stimulus intensity of 15 V the predicted and experimental levels of facilitation/augmentation were not significantly different (compare Figure 3B, filled squares with dashed line) indicating no inhibition of transmitter release even though relatively more transmitter was being liberated from CMT preparations compared to CST preparations (filled squares in Figure 4A). When the stimulus intensity was increased there was a progressive decrease in facilitation/augmentation (in Figure 3B compare filled squares, 15 V with filled circles, 20 V and filled triangles, 30 V) as the relative amount of transmitter release increased. Introduction of idazoxane (1 μM) to the bathing solution increased the level of experimental facilitation/augmentation so that there was no significant difference between predicted and experimental facilitation/augmentation at stimulus intensity of 20 V (Figure 3D, filled circles). At stimulus intensity of 30 V however (Figure 3D, filled triangles) there was very little facilitation/augmentation even after bathing the preparation with idazoxane (1 μM) for more than 50 min. The disparity between expected and observed facilitation/augmentation increased and was shown to be partly due to α_2-adrenoceptor mediated autoinhibition (Figure 4B compare open and closed squares). The discrepancy between experimental and predicted facilitation/augmentation when stimulating at 30 V (Figure 3B,D) was probably due to depletion of available vesicles and/or due to non-linear summation (Figure 4).

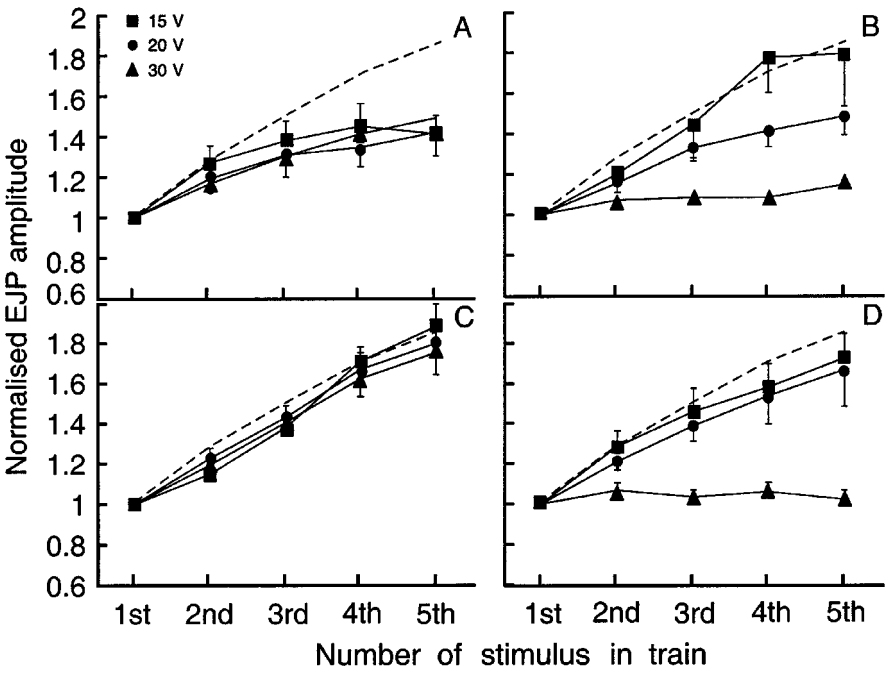

Figure 3 Facilitation of EJP amplitude at different stimulus intensities from CST and CMT preparations. In (A) and (B) no drug was present in the bathing solution while in (C) and (D) idazoxane (1 μM) was added to the bathing solution. (A) and (C) are results obtained from CST preparations while (B) and (D) are results obtained from CMT preparations. The normalized amplitude of EJPs with respect to the first EJP in a train is plotted. The symbols indicate the voltage used to stimulate the sympathetic axons: ■ 15 V; ● 20 V; ▲ 30 V. Symbols with a value greater than 1 on the ordinate indicates facilitation. Dashed lines indicate the predicted level of facilitation assuming a time constant of decay in facilitation of 6 s. The difference between the predicted and experimental level of facilitation was used as a measure of the depression of EJP amplitude (see Figure 4). EJPs were evoked by trains of five stimuli at 1 Hz.

Discussion

Effect of CMT on the density of innervation

Although the enhancement of transmitter release from sympathetic varicosities undergoing withdrawal from CMT is mainly produced by an increase in the probability of transmitter release (Lavidis, 1995b), an increase in the density of sympathetic varicosities may also contribute to this enhancement. In the present investigation, we have observed a significant increase (31%) in the density of sympathetic varicosities of CMT vasa deferentia. This increased density may contribute to part of the enhancement of transmitter release observed after withdrawal from 7 to 9 days of CMT.

Further support for an increase in innervation of CMT preparations has come from studying the relationship between stimulus intensity and EJP amplitude. In CST preparations, increasing stimulus intensity beyond 15 V did not alter the amplitude of EJPs or reduce the inhibitory effect of morphine on transmitter release. This was expected since morphine does not affect the propagation of the nerve terminal impulse (Cunnane & Evans, 1988; Lavidis, 1995a). In CMT preparations, the mean amplitude of EJPs increased with increasing stimulus intensity over the range 15–30 V. Possible reasons for the difference in the EJP versus stimulus intensity relationship between CST and CMT preparations are: growth of new axons in CMT preparations, with the new axons having a higher threshold for initiating an action potential; an increase in coupling between smooth muscle cells during CMT. In the present study we have demonstrated an increase in the density of varicosities distributed on the surface and deeper regions of CMT preparations. This increase was achieved by an increase in the branching complexity of sympathetic axons rather than a decrease in the spacing between varicosities.

Interaction between opioid receptors and α_2-adrenoceptors

Although the interaction between opioid and α_2-adrenoceptors has been previously studied in brain tissues (Schoffelmeer *et al*., 1986; Limberger *et al*., 1988b; Illes & Norenberg, 1990) and arteries (Ramme *et al*., 1986; Bucher *et al*., 1992), the level at which this interaction occurs has not been determined (Bucher *et al*., 1992). Possible levels of interaction include the receptor (Illes *et al*., 1980; Ramme *et al*., 1986; Schoffelmeer *et al*., 1986; Illes & Norenberg, 1990) or the calcium channels, or the proteins associated with vesicle fusion (Lipscombe *et al*., 1989; Allgaier *et al*., 1989). Studies of the ability of yohimbine to antagonize the anti-nociceptive actions of morphine have been inconclusive. Soloman & Gebhart (1988) have shown that yohimbine does not modify the anti-nociceptive action of morphine while Camarata & Yaksh (1985) produced evidence to the contrary.

Indirect evidence indicates that opioid receptors and α_2-adrenoceptors affect voltage gated calcium channels to reduce the amount of Ca^{2+} entering the terminal during nerve stimulation. This is likely since procedures which are thought to increase the amount of calcium entering the terminal

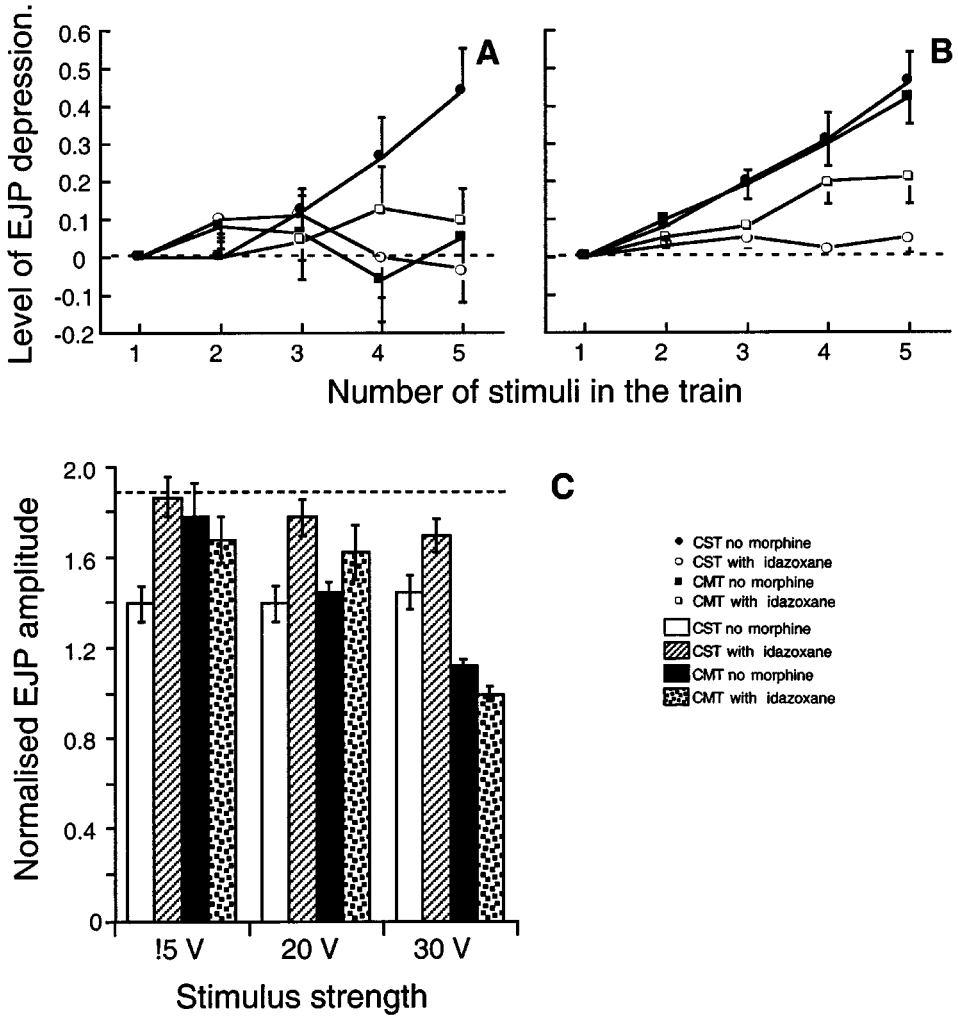

Figure 4 Effect of idazoxane on depression of EJP amplitude in CST and CMT preparations. The level of depression of EJPs during stimulation by trains of impulses (5 at 1 Hz). (A) stimulus intensity of 15 V and (B) stimulus intensity of 20 V. In (A) and (B) symbols indicate the mean level of depression while positive going and negative going lines indicate plus or minus s.e.mean (see key for explanation of each symbol). Dashed line indicates no depression. (C) normalized EJP amplitude of the 5th EJP amplitude with respect to the 1st EJP amplitude is indicated on the ordinate while on the abscissa tissue treatment is indicated. Three levels of stimuli intensities are indicated on the abscissa (15, 20 and 30 V). Bars indicate the mean normalized EJP amplitude while lines on top of bars indicate $\pm$ s.e.mean ($n > 7$). The different bar patterns in (C) indicate different experimental conditions as indicated by the key.

during nerve stimulation, or increase in residual calcium following stimulation with trains of impulses, reduce the effect of morphine and clonidine (Bennett & Lavidis, 1980; Illes *et al*., 1980; Alberts *et al*., 1981; Lavidis, 1995a). Calcium imaging studies of the residual intravaricosity calcium levels following single impulses and high frequency trains of impulses have yielded conflicting results about the action of α_2-adrenoceptor agonists (Brain & Bennett 1997; O'Connor *et al*., 1999). O'Connor *et al*. (1999) have shown that clonidine (1 μM) does not affect the residual calcium levels of varicosities following single nerve stimulation but does reduce the residual calcium levels following high frequency stimulation. This is in complete contradiction with the expected result since clonidine is most effective in inhibiting transmitter release during stimulation by single impulses and least effective during stimulation with trains of nerve impulses. The calcium imaging data of O'Connor *et al*. (1999) indicate that the clonidine-induced reduction of transmitter release is not produced by a reduction in calcium influx. It is possible that the measurements of the residual calcium levels within varicosities following nerve stimulation poorly reflect the calcium levels at the active zone that are thought to be responsible for triggering transmitter release. Alternatively, clonidine may induce an inhibition of transmitter release by affecting the phosphorylation state of vesicular associated proteins such as synapsin 1. Such a mechanism has been proposed for the action of morphine on transmitter release (Nah *et al*., 1993).

Further support for a common point of interaction between opioid receptors and α_2-adrenoceptors has come from the CMT experiments. Acute withdrawal from CMT increases the probability of transmitter release from known numbers of sympathetic varicosities of the mouse vas deferens, possibly by enhancing calcium entry during nerve

stimulation (Lavidis, 1995b), or by changing the phosphorylation state of vesicular associated proteins (Nah *et al*., 1993). In the present study we have demonstrated that enhancement of transmitter release from sympathetic varicosities following the development of tolerance to morphine (Einstein & Lavidis, 1984b; Lavidis, 1995b) underlies the decrease in effectiveness of morphine and clonidine.

Effect of CMT on α_2-adrenoceptor mediated autoinhibition of transmitter release during high frequency nerve stimulation

In CST preparations, stimulation with trains of impulses (1 Hz) leads to an increase in the amplitude of EJPs from impulse to impulse (facilitation, Burnstock *et al*., 1964). Residual calcium bound to a calcium receptor has been used to explain facilitation of transmitter release during stimulation with trains of impulses (Katz & Miledi, 1968). In sympathetic varicosities, facilitation/augmentation declines with a time constant of 6 s (Bennett & Florin, 1975; Einstein & Lavidis, 1984a,b). A concurrent depression in transmitter release is also present (McCulloch *et al*., 1985), which declines exponentially with a time constant of 4 s (unpublished observation). In CST preparations, the difference between experimental and theoretical levels of facilitation in EJP amplitude observed in this study was therefore due to the concurrent depression. When idazoxane was added to the bathing solution, the depression was abolished, unmasking the full extent of facilitation/augmentation, and demonstrating that activation of presynaptic α_2-adrenoceptors was responsible for most of the depression in EJP amplitude.

In CMT preparations withdrawn from morphine, the lack of difference between experimental and theoretical levels of facilitation in transmitter release when the stimulus intensity was 15 V indicated a decrease in α_2-adrenoceptor mediated inhibition. This occurred even though the relative level of transmitter release in CMT preparations was greater than for CST preparations. Increasing the stimulus intensity of CMT preparations increased the level of transmitter released and depression. The depression was inhibited by idazoxane when the stimulus intensity was 20 V indicating that it was predominantly due to α_2-adrenoceptor mediated autoinhibition. The depression observed when the stimulus intensity was 30 V could not however be reduced by idazoxane indicating that it was not induced by α_2-adrenoceptor activation but possibly by depletion of available vesicles or/and non-linear summation.

Conclusion

Although the present study has demonstrated an apparent decrease in the efficacy of both morphine and clonidine to inhibit transmitter release from sympathetic varicosities during CMT, the primary cause of this effect was shown to be a significant increase in transmitter release from sympathetic varicosities. This increase in transmitter release is not only produced by an increase in probability of transmitter release from sympathetic varicosities (as has been reported previously, Lavidis 1995a,b) but as was shown in the present study by an increase in the density of innervation. In the next study we aim to investigate the possible role of neurotrophins in the changes observed in the present study.

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