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Electrophysiological and behavioral effects of Tyr-D-Arg-Phe-Sar on locus coeruleus neurons of the rat

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Abstract

The effect of Tyr-D-Arg-Phe-Sar (TAPS), a μ-selective tetrapeptide analog of dermorphin, was studied in the rat both in vitro, using slices of the locus coeruleus, and in vivo, after microinjection into the locus coeruleus. In electrophysiological studies, TAPS (1–100 nM) was able to inhibit spontaneous firing, cause hyperpolarization of the membrane potential and reduce the input resistance of neurons of the locus coeruleus, suggesting that there was an effect on the potassium channels. Based on the inhibition of the spontaneous firing rate, the average IC_{50} for TAPS was calculated to be 1.9 nM, a value lower than that reported for dermorphin or morphine. The TAPS-induced effects were antagonized by naloxone, with a dissociation equilibrium constant of 1.96 ± 0.14 nM. The results indicate that TAPS binds to μ-opioid receptors on the cell membrane of neurons of the locus coeruleus to cause its inhibitory actions. In behavioral study, TAPS was microinjected bilaterally via chronically implanted cannulae into the locus coeruleus of non-anesthetized rats and its effects on locomotor activity determined. TAPS, at concentrations of 1 μM and 10 μM, but not of 0.1 μM, induced hypolocomotion/sedation and the effect was significantly reversed by naloxone (5 mg/kg i.p.). Taken together, these data suggest that TAPS has an inhibitory effect on neurons of the locus coeruleus and produces hypolocomotive/sedative effects in vivo. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Tyr-D-Arg-Phe-Sar; Locus coeruleus; Intracellular recording; Locomotor activity

1. Introduction

The opiates or opioid peptides are known to be involved in the control of motor activity. A systemic morphine administration has shown to produce dose- and time-related biphasic effects on spontaneous locomotor activity in rats (Babbini and Davis, 1972; Buxbaum et al., 1973). Low to moderate doses of morphine (i.e., 1.0–5.0 mg/kg) produce stimulation of activity, whereas higher doses (i.e., 10–40 mg/kg) produce a biphasic effect consisting of an initial depression of activity, followed by a period of hyperexcitability. Recent studies on rats, using several μ-opioid receptor agonists, also suggest that central microinjection of several μ-opioid receptor agonists results in biphasic effects on locomotor activity (Meyer and Meyer, 1993a,b; Meyer et al., 1995). The mechanism or site of action for the biphasic response to μ-opioid receptor agonists is yet to be determined.

The locus coeruleus is a compact group of norepinephrine-containing cell bodies located near the floor of the fourth ventricle at the upper border of the pons, and projections from this small pontine nucleus give rise to more than half the noradrenergic neurons in the brain (Amaral and Sinnamon, 1977). The noradrenergic neurons of the locus coeruleus show both a high opioid-receptor density (Lewis et al., 1985; Mansour et al., 1986) and high opioid-receptor mRNA expression (Mansour et al., 1995); electrophysiological studies indicate that opioid receptors in the locus coeruleus are of the μ type (Williams and North, 1984; North, 1986). Moreover, immunocytochemical and immunofluorescent studies show that opioid peptide-containing nerve terminals innervate neurons of the locus coeruleus (Finley et al., 1981; Léger et al., 1983). The locus coeruleus is also involved in central opiate actions and it has been shown that opiates, acting via μ-opioid receptors, hyperpolarize neurons of the locus

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coeruleus (Williams and North, 1984; Aghajanian and Wang, 1987; Chiu et al., 1990, 1993).

Tyr-D-Arg-Phe-Sar (TAPS, Sar = *N*-methylglycine) is a μ-selective tetrapeptide analog of dermorphin that has an especially high affinity for opioid receptors, being 25 or 3 times as potent as morphine or dermorphin, respectively (Sato et al., 1987). In antinociceptive tests, it is about 6 times as potent as dermorphin (Sasaki et al., 1984; De Castiglione and Rossi, 1985; Sato et al., 1987). Our previous study demonstrated that dermorphin binds to μ-opioid receptors on the cell membrane of neurons of the locus coeruleus, leading to the opening of the inward-going rectification potassium channels and resulting in the observed hyperpolarization of the membrane (Chiu et al., 1990). We also found the depressant effects of dermorphin on neurons of the locus coeruleus to be more potent than those of morphine (Chiu et al., 1990). Moreover, functional studies have demonstrated that the locus coeruleus is one of the major neural sites involved in sedation (De Sarro et al., 1987; Correa-Sales et al., 1992; Pertovaara et al., 1994). The aims of the present investigation were therefore to examine the action of TAPS on neurons of the locus coeruleus using intracellular recording analysis techniques and to determine whether microinjection of TAPS into the locus coeruleus would affect locomotor activity in rats.

2. Materials and methods

2.1. In vitro electrophysiological studies

2.1.1. Preparation and maintenance of slices of locus coeruleus

The methods used to prepare and maintain slices of rat locus coeruleus were similar to those previously described (Chiu et al., 1990, 1993, 1995). Male Sprague–Dawley rats (120–200 g) were sacrificed and their brains rapidly removed. A block of tissue containing the pons was excised and attached to a small Plexiglass stage using cyanoacrylate glue; an agar block, placed next to the tissue, helped to support it during sectioning. The tissue was then submerged in oxygenated artificial cerebrospinal fluid (artificial CSF), maintained at 3–5°C, in the well of a Lancer 1000 vibratome. Several 300–350-μm thick coronal sections of the pons were cut, and a slice containing a cross-section through the caudal end of the locus coeruleus mounted in the recording chamber and allowed to equilibrate for 1 h; the slice was completely submerged in a heated (33–34°C) flowing (2.3 ml/min) solution with the following millimolar composition: NaCl 126, KCl 2.5, NaH_2PO_4 1.2, $NaHCO_3$ 26.2, $MgCl_2$ 1.3, $CaCl_2$ 2.4, glucose 11.1, gassed with 95% O_2/5% CO_2, and was viewed from above using a dissection microscope. In the trans-illuminated slice, the locus coeruleus was seen as a translucent area lying on the lateral aspect of the periventricular gray, below the fourth ventricle.

2.1.2. Intracellular recording

Intracellular recording from neurons of the locus coeruleus was performed using sharp microelectrodes, filled with 2-M KCl, with a DC tip resistance of 40–70 MΩ. The recording microelectrodes were inserted into the locus coeruleus under visual control. Intracellular potentials were recorded using an amplifier with an active bridge circuit, permitting current injection through the recording electrode (WPI M707). Current and voltage traces were displayed on a storage oscilloscope (Textronix 5113) and a rectilinear pen recorder (Gould 2400). Input resistance was measured by passing hyperpolarizing constant current pulses of sufficient duration to fully charge the membrane capacitance and reach a steady-state voltage deflection.

2.1.3. Perfusion of solutions and drugs

A valve system was used to switch the perfusion solution between control artificial CSF to drug-containing CSF. The period required for test solutions to reach the chamber was known and ranged from 25–35 s. The drugs used were naloxone hydrochloride (Sigma) and TAPS (Bachem). Numerical data are expressed as the mean $\pm$ the standard error of the mean (S.E.M.).

2.2. In vivo behavioral studies

2.2.1. Animals

Male Sprague–Dawley rats, weighing 300–350 g, were obtained from the National Science Council, Taiwan, Republic of China. The rats were individually housed and maintained on a 12:12-h light:dark cycle (lights on at 0600), with food and water provided ad libitum. The animals were tested in the light phase between 1000–1700.

2.2.2. Surgery

The rats were anesthetized with 40 mg/kg i.p. pentobarbital and placed on a stereotaxic instrument. Two 23-gauge stainless steel thin wall cannulae (15-mm long) were implanted bilaterally into the locus coeruleus with the cannula tip being aimed at the dorsal surface of the locus coeruleus at the following coordinates: A.P. -1.0 mm from lambda, M.L. ± 1.3 mm from midline and D.V. -6.4 mm below the skull surface; the tooth bar was at -3.3 mm. The guide cannulae were fixed to the skull using three stainless screws and dental acrylic cement and a stainless steel stylet inserted into each cannula to maintain patency. After the operation, the rats received penicillin, then were allowed to recover in individual cages for 7–10 days.

2.2.3. Intracerebral drug administration

Animals received bilateral microinjections of saline or different doses of TAPS into the locus coeruleus before the assessment of their motor functions. The animal was awake

and held gently by the experimenter during injection. The drug was microinjected into the locus coeruleus through a 30-gauge stainless steel injection cannula with the injection needle bent so that, when inserted into the guide cannula, the needle tip would protrude 1.5 mm beyond the guide cannula tip. The injection was performed bilaterally and the volume of 1 μl using a 10-μl Hamilton microsyringe connected to a length of PE-20 polyethylene tubing. The efficacy of injection was monitored by observing the movement of a small air bubble through the tubing. Post injection on each side for 1 min, the injection cannula was then left in place for an additional 30 s to minimize flow of the drug solution back up into the injection track. TAPS was dissolved in 0.9% isotonic saline immediately before use.

2.2.4. Assessment of motor activity

The activity monitor has been described in detail elsewhere (Lee et al., 1988). In brief, it consisted of two activity chambers (Coulbourn Instrument, PA), approximately 16 in. square, with 16×16 horizontal and vertical infrared sensors, used to localize the position of the animal and to quantify their motor activities. The measurements include horizontal activity, vertical activity and stereotyped behavior. Horizontal activity is measured by total number of beam breaks in an X–Y plane recorded every 10 ms. Vertical activity is similarly measured in an X–Z plane (vertical plane). On the other hand, whenever the animal starts grooming or scratching and stops for 1 s, such activity is considered as a single burst of stereotypic activity. These bursts are totalled and labelled as counts of stereotypic behavior. Only one animal at a time was placed in each activity chamber during the measurement period. These activities were measured over two 20-min periods, starting immediately after, or 50 min after, intracerebral drug administration.

2.2.5. Histology

Following behavioral testing, the animals were sacrificed by decapitation and the brains removed. For histological examination of cannula and needle placement in the locus coeruleus, the brains were frozen-sectioned in a cryostat and checked individually. Twenty-micron thick sections, taken at 40-μm intervals throughout the locus coeruleus, were mounted on slides and stained with Evan's blue. Animals were accepted for data analysis only when both needle placements were located within the locus coeruleus according to the atlas of Paxinos and Watson (1986).

2.2.6. Statistics

Eight to ten animals were used in each independent treatment group. The animals were treated and tested only once. The behavioral data were analyzed with a one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test. P values equal to, or less than, 0.05 were judged to be statistically significant.

3. Results

3.1. In vitro electrophysiological studies

3.1.1. Membrane properties of locus coeruleus neurons

Electrophysiological properties were examined in a total of 46 locus coeruleus neurons with stable intracellular impalement. All locus coeruleus neurons included in this study showed spontaneous activity, the frequency of spontaneous firing ranged from 0.1 to 4.3 Hz (1.9 ± 0.1 Hz, $n = 46$). The pattern of locus coeruleus neuron spontaneous firing recorded in each slice was very regular, i.e., the interspike interval was remarkably uniform. The neurons had resting membrane potentials of -42 to -65 mV (-53.7 ± 0.8 mV, $n = 41$) and apparent input resistances of 112 to 360 MΩ (182 ± 7 MΩ, $n = 46$).

3.1.2. Effects of TAPS

TAPS (1–100 nM) reversibly decreased the firing rate of all locus coeruleus neurons tested. Although the neurons varied in their sensitivity to TAPS, inhibition of the firing rate, hyperpolarization of membrane potential and reduction in input resistance depended on concentration (Fig. 1). Inhibition of firing rate was the most sensitive parameter and showed a marked change at lower concentrations; it was seen in all locus coeruleus neurons tested, with an IC_{50} of 1.9 nM. At the concentrations of 1- and 3-nM TAPS, which respectively produced an inhibition of firing rate of 25.6% ($n = 5$) and 78.1% ($n = 8$, including 1 cell in which firing was completely suppressed), slight hyperpolarization and a small reduction in input resistance were seen. Higher concentrations of TAPS (10–100 nM) resulted not only in complete inhibition of spontaneous

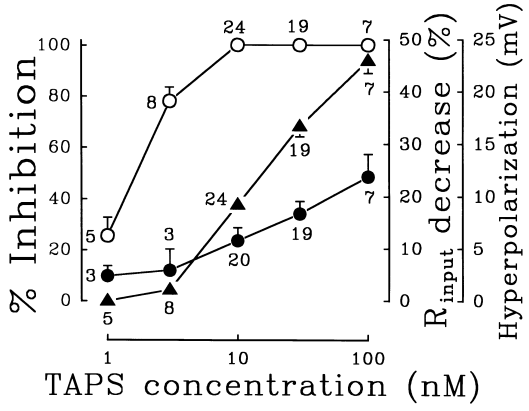

Fig. 1. Dose-dependent effects of TAPS on the firing rate (○), membrane potential (▲) and input resistance (●) of neurons of the locus coeruleus. The vertical bars represent the S.E.M. for the number of neurons indicated.

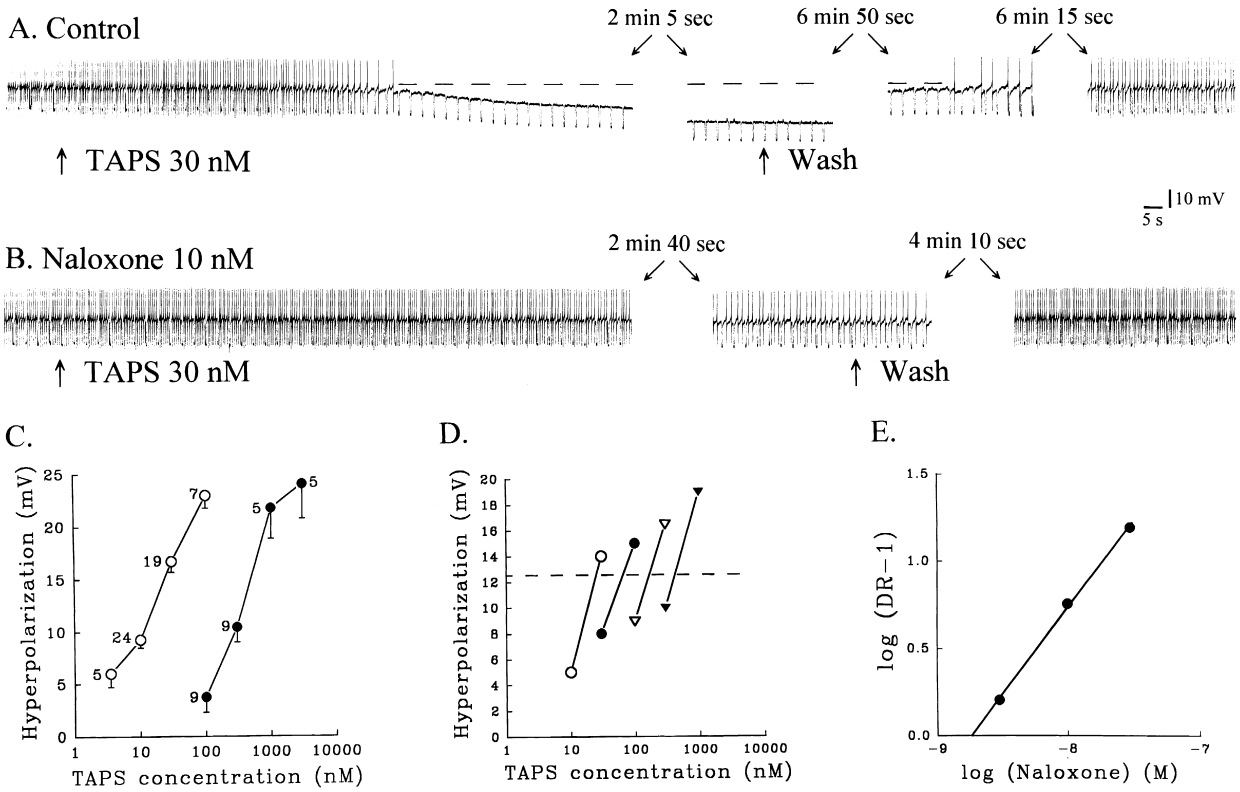

Fig. 2. Naloxone antagonism of TAPS-induced hyperpolarization. (A) Superfusion with 30-nM TAPS caused complete inhibition of the firing rate, hyperpolarization of the membrane potential (20 mV) and a decrease in input resistance (17.5%). The broken horizontal line indicates −52 mV. (B) In the presence of 10-nM naloxone, 30-nM TAPS produced no hyperpolarization. However, inhibition of spontaneous firing still occurred, though it was incomplete (63.6% inhibition). (C) Dose–response curves for TAPS in the presence or absence of naloxone: control (○); naloxone 10 nM (●). The vertical bars represent the S.E.M. for the number of neurons indicated. (D) Dose–response curves for TAPS in the presence of different concentrations of naloxone: control (○); naloxone 3 nM (●); 10 nM (▽) and 30 nM (▼). The data were obtained by recording from a single neuron. (E) Schild plot of the data shown in (D), at a response level of 12.5 mV. The dissociation equilibrium constant was 1.82 nM in this neuron.

firing in all neurons tested, but also in membrane hyperpolarization and a decreased input resistance (Fig. 2A). Different neurons showed considerable variation in both the extent of hyperpolarization and the reduction of input resistance induced by the same concentration of TAPS. For example, in response to 10-nM TAPS, the amplitude of hyperpolarization and the reduction of input resistance ranged from 2.5 to 15 mV (9.3 ± 0.8 mV, $n = 24$) and from 1.8 to 41.8% ($11.6 \pm 2.5\%$, $n = 20$), respectively. At 100 nM, TAPS produced complete inhibition of firing of all neurons tested ($n = 7$), together with a 23 mV hyperpolarization (range 18.5–28 mV, $n = 7$) and a 23.9% reduction in input resistance (range 3.7–37.4%, $n = 7$).

3.1.3. Effect of naloxone on reversing the hyperpolarization caused by TAPS

Naloxone both prevented opioid-induced hyperpolarization when it was applied prior to the agonist (Fig. 2B), and terminated an ongoing hyperpolarization response when added to the agonist-containing solution. Perfusion of locus coeruleus neurons with naloxone (10 nM) produced no significant change in membrane potential or input resistance, yet, a small increase in firing rate was occasionally observed. Pretreatment with naloxone (10 nM) before changing the perfusate to a mixture of 10-nM naloxone and TAPS at various concentrations (100–3000 nM) resulted in a parallel, dose-related shift to the right of the TAPS concentration–response curve (Fig. 2C). In another set of experiments, TAPS dose–response curves, constructed in the presence of increasing naloxone concentrations while recording from a single neuron, were used to determine the dissociation equilibrium constant (K_d) for the antagonist. An example is shown in Fig. 2D; naloxone (3–30 nM) produced a parallel, dose-related shift to the right of the TAPS dose–response curve. The 'dose ratios', calculated by dividing the concentration of TAPS required to produce a given hyperpolarization in the presence of naloxone by that required to produce the same hyperpolarization in the absence of naloxone, were used to construct the Schild plots (Fig. 2E), described by Williams and North (1984). These experiments gave a K_d value of 1.96 ± 0.14 nM ($n = 5$) for naloxone antagonism of the TAPS-induced hyperpolarization.

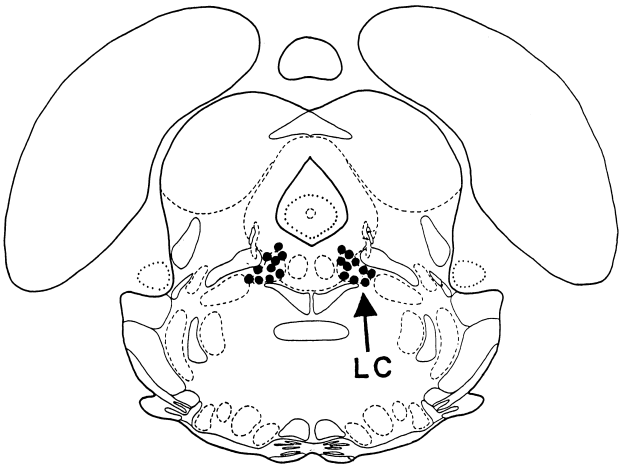

Fig. 3. Schematic drawing of a coronal section of the rat brain, showing the centers of the histologically verified injection sites (black dots) in the locus coeruleus (LC).

3.2. In vivo behavioral studies

3.2.1. Verification of injection sites

Fig. 3 shows the central points of the histologically verified microinjection sites, all of which were within, or adjacent to, the locus coeruleus.

3.2.2. Horizontal activity

Fig. 4 shows the horizontal activity elicited by bilateral microinjection of TAPS into the locus coeruleus. During the first 20-min test period (0–20 min post microinjection), TAPS produced a significant dose-dependent effect on the horizontal activity ($F(3,31) = 8.66$, $P < 0.0005$; Fig. 4). No significant effect was seen at the lowest dose tested (0.1 μM) compared with the effect of saline, but a marked reduction was seen at the two higher TAPS doses studied (1 μM and 10 μM) ($P < 0.01$ and 0.005, respectively). Using the dose of 10-μM TAPS, the decrease in horizontal activity was significantly ($P < 0.01$) reversed by naloxone (5 mg/kg i.p.); naloxone alone (5 mg/kg i.p.) had no significant locomotive effect (Fig. 4). During the second 20-min test period (50–70 min post microinjection), no significant difference was observed in suppression of horizontal movement compared to controls ($P > 0.05$) at the two lower doses (0.1 μM and 1 μM), while a significant effect ($P < 0.05$) was seen at 10 μM TAPS.

3.2.3. Vertical activity

Fig. 5 illustrates the significant differences between animals treated with the three TAPS dose levels and the vehicle controls ($F(3,31) = 9.2$, $P < 0.0005$) during the first 20-min test period (0–20 min post microinjection). When compared with the effect of saline, the lowest dose (0.1 μM) had no significant effect, while both the higher doses (1 μM and 10 μM) caused a significant decrease in vertical activity ($P < 0.01$ and 0.005); that produced by 10-μM TAPS was significantly ($P < 0.01$) reversed by naloxone (5 mg/kg i.p.). During the second 20-min test period (50–70 min post microinjection), only the highest dose of TAPS (10 μM) produced a significant decrease in vertical activity ($P < 0.05$).

3.2.4. Stereotypy

Fig. 6 shows the significant effects of the three dose levels in comparison with the vehicle controls ($F(3,31) = 7.12$, $P < 0.001$) during the first 20-min test period. Again, the 0.1 μM TAPS-treated group showed no significant suppression of stereotypy, while both the 1 μM- and 10 μM-TAPS treated groups showed significant suppression when compared to the vehicle controls ($P < 0.05$ and 0.005). Naloxone (5 mg/kg i.p.) completely reversed the

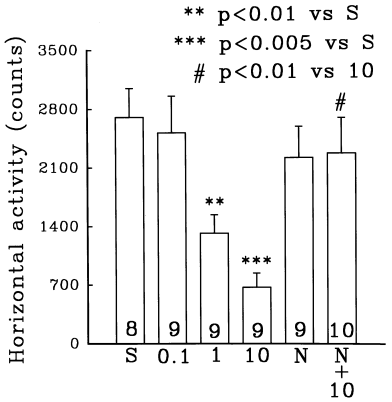

Fig. 4. Effects on horizontal activity following bilateral microinjection of saline (S) or TAPS (0.1, 1 and 10 = 0.1–10 μM) into the locus coeruleus and i.p. injection of naloxone (N; 5 mg/kg). Locomotor activity was measured for 20 min immediately following i.p. (and/or intracerebral) drug administration. Each column represents the mean (±S.E.M.) of results obtained in 8–10 rats, the numbers being shown in the bars.

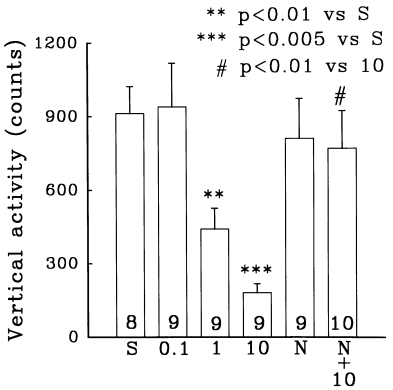

Fig. 5. Effects on vertical activity following bilateral microinjection of saline (S) or TAPS (0.1, 1 and 10 = 0.1–10 μM) into the locus coeruleus and i.p. injection of naloxone (N; 5 mg/kg). Locomotor activity was measured for 20 min immediately following i.p. (and/or intracerebral) drug administration. Each column represents the mean (±S.E.M.) of results obtained in 8–10 rats, the numbers being shown in the bars.

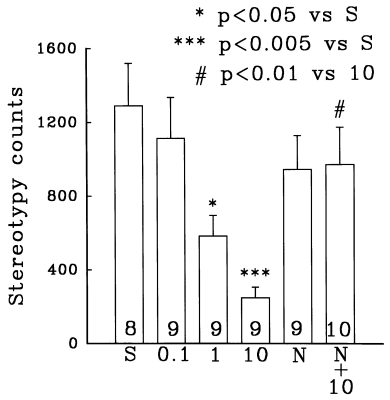

Fig. 6. Effects on stereotypy following bilateral microinjection of saline (S) or TAPS (0.1, 1 and 10 = 0.1–10 μM) into the locus coeruleus and i.p. injection of naloxone (N; 5 mg/kg). Locomotor activity was measured for 20 min immediately following i.p. (and/or intracerebral) drug administration. Each column represents the mean (±S.E.M.) of results obtained in 8–10 rats, the numbers being shown in the bars.

effect produced by 10-μM TAPS. Again, during the second 20-min test period, only the highest dose of TAPS (10 μM) had a significant suppressive effect on stereotypy ($P < 0.05$).

4. Discussion

In this study, we found that TAPS binds to μ-opioid receptors on the cell membrane of neurons of the locus coeruleus, resulting in hyperpolarization of the membrane and a reduction in the input resistance of the neurons. These findings are consistent with those of previous studies which indicate that opiates, acting via μ-opioid receptors, reduce neuronal excitability by opening the inward-going rectification potassium channels and producing hyperpolarization (Chiu et al., 1990, 1993; Nestler et al., 1994). In a previous study, we demonstrated that the average IC_{50} for dermorphin in inhibiting locus coeruleus neuronal activity is 7 nM (Chiu et al., 1990) and that the inhibitory effect of dermorphin on neurons of the locus coeruleus is more potent than that of other opioids, such as morphine, normorphine, met-enkephalin, β-endorphin and [D-Ala^2-NMePhe^4-Gly-ol^5]enkephalin (see Discussion section of Chiu et al., 1990). A comparison of the ability of TAPS and dermorphin to inhibit locus coeruleus neuronal firing showed TAPS to be 3.7 times as potent. The present results are compatible with previous findings that the affinity of TAPS for μ-opioid receptors is 25 or 3 times greater than that of morphine or dermorphin, respectively (Sato et al., 1987), that, in antinociceptive tests, TAPS is about 6 times as potent as dermorphin (Sasaki et al., 1984; Sato et al., 1987) and that s.c. administered TAPS is 21 times more potent than morphine in the mouse tail-pressure test (Sasaki et al., 1984). Additionally, we have also found TAPS to be more effective on neurons of the locus coeruleus than the other naturally occurring opioid tetrapeptides, such as Tyr-D-Arg-Phe-Lys-NH_2 (DALDA, a dermorphin analog), Tyr-Pro-Phe-Pro-NH_2 (morphiceptin, a tetrapeptide fragment of the milk protein β-casein), Tyr-Pro-Trp-Thr (hemorphin-4, derived from the β-chain of hemoglobin), Tyr-Pro-Leu-Gly-NH_2 (Tyr-MIF-1, isolated from the brain, MIF = melanocyte-stimulating hormone release inhibiting factor = Pro-Leu-Gly-NH_2) and Tyr-Pro-Trp-Gly-NH_2 (Tyr-W-MIF-1, isolated from the brain) (unpublished observations, see also Yang and Chiu, 1997). The high potency of TAPS may be explained by the following reasons: (1) it is thought that the introduction of D-amino acids into peptides can provide resistance to degrading enzymes (Pert et al., 1976; Roemer et al., 1977; Sato et al., 1987), and (2) in terms of the structure–activity relationship for the N-terminal tetrapeptide of dermorphin, the substitution of the D-Ala^2 or Gly^4 residues with D-Arg or sarcosine (*N*-methylglycine), respectively, markedly enhances the potency of the tetrapeptide (Sasaki et al., 1984; Sato et al., 1987). From the clinical point of view, TAPS has several advantages over other opiates or opioid peptides. Firstly, despite its marked opioid action, TAPS clearly results in less dependence than morphine; its abrupt withdrawal produces only a slight loss of body weight, while naloxone-precipitated withdrawal signs are less intense than in morphine-treated rats (Nakata et al., 1986). Secondly, unlike other opioids or opiates, TAPS does not produce respiratory depression, but rather, stimulates the ventilatory minute volume after i.c.v. administration (Paakkari et al., 1993). Thirdly, it is noteworthy that TAPS also elicits antinociceptive effects after i.v., or even p.o., administration in the rat (Paakkari et al., 1993). Taken together, these results suggest a possible clinical use for this opioid tetrapeptide (Sato et al., 1987; Paakkari et al., 1993).

This study also demonstrated that the effects of TAPS on neurons of the locus coeruleus appear to be specifically mediated through opioid receptors, since naloxone was effective in reversing the TAPS-induced hyperpolarization. The observation that naloxone was able to counteract the inhibitory effect of TAPS on neurons of the locus coeruleus is consistent with previous reports demonstrating that naloxone competitively reverses the effect of opioid substances, with a dissociation equilibrium constant in the range of 1.5–2 nM (Williams and North, 1984; McFadzean et al., 1987). Our work shows that naloxone antagonized the actions of TAPS, with a dissociation equilibrium constant of about 1.96 nM, within the range used to define the μ-opioid receptor (0.5–3 nM, North, 1986) and much smaller than the antagonistic K_d values for naloxone on δ- and κ-receptors (30 and 15 nM, respectively, North, 1986). These results suggest that the hyperpolarization caused by TAPS is mediated by μ-opioid receptors.

Since TAPS had a strong inhibitory effect on neurons of the locus coeruleus in vitro, we assessed whether TAPS

also exerted effects on locomotor activity. Our data show that, on bilateral microinjection into the locus coeruleus, TAPS produces dose-dependent hypolocomotion/sedation. During the first 20-min test period (0–20 min post microinjection), 10-μM TAPS elicited a significant suppressive effect on horizontal activity, vertical activity and stereotypy, the effects lasting for up to 70 min. In rats, μ-opioid receptor agonists (i.c.v.) are reported to typically elicit a biphasic effect on locomotor activity, with initial suppression of activity, followed by excitation (Meyer and Meyer, 1993a,b; Meyer et al., 1995). In contrast, in this study, TAPS only produced a monophasic effect, that of hypoactivity, without the later activation phase seen with other opiates or opioid peptides. The difference in behavioral profile between TAPS, microinjected into the locus coeruleus, and other opioids, injected i.c.v., may be explained by the biphasic effect being perhaps due to local diffusion of these opioid peptides to various brain regions (Meyer and Meyer, 1993b; Meyer et al., 1994). In this respect, it has been established that opiates and opioid peptides induce monophasic hypoactivity if microinjected into the periaqueductal gray and unidirectional excitation via the striatum (Dauge et al., 1988; Jenck et al., 1988), and it is therefore understandable that microinjection of TAPS into the locus coeruleus would result in monophasic hypolocomotion.

Consistent with the present finding, Garzón et al. (1995) have indicated that the unilateral microinjection of morphine or morphiceptin (a specific μ agonist) into the locus coeruleus of the cat produces a significant hypnotic/sedative response. Interestingly, microinjection of α_2-adrenoceptor agonists into the locus coeruleus results in similar physiological effects. For example, bilateral microinjection of medetomidine, an α_2-adrenoceptor agonist, into the locus coeruleus produces dose-dependently hypolocomotion/sedation (Pertovaara et al., 1994). By recording the electrocorticogram, De Sarro et al. (1987) also demonstrated that injection of the α_2-adrenoceptor agonist, clonidine, into the locus coeruleus produces powerful behavioral sedative effects, accompanied by electrocortical slow-wave sleep. This phenomenon, that microinjection of α_2-adrenoceptor agonists or μ-opioid receptor agonists into the locus coeruleus produces identical behavioral effects, can be explained by previous electrophysiological findings which demonstrate that agonists of both the above receptor types open the inwardly rectifying potassium channels, resulting in the observed membrane hyperpolarization of neurons of the locus coeruleus (Williams et al., 1985, 1988; Aghajanian and Wang, 1987; Chiu et al., 1993, 1995).

Regarding the reasons for the different effective concentrations seen in the in vitro and in vivo experiments, i.e., the lowest TAPS dose (0.1 μM) had no inhibitory effect on the rat's behavior, but resulted not only in complete inhibition of the spontaneous firing of all neurons tested, but also in significant hyperpolarization of the membrane, this might be explained as in the following. First, it is quite common in the literature that the actual drug concentration of microinjection in the in vivo experiments is uncertain due to diffusion and degradation of drugs in the extracellular milieu (e.g., see Aston-Jones and Siggins, 1995). Secondly, the involvement of extrinsic connections in vivo gives a more complicated picture than the simple intrinsic interactions seen in vitro (Smialowska et al., 1996). In the in vivo situation, the mechanism of sedation has been proposed as such that inhibition of locus coeruleus firing reduces the amount of norepinephrine released from axon terminals in the thalamus and cerebral cortex, and thereby reduces sensory information reaching the cortex and making the cortex less receptive to sensory information (Ruffolo et al., 1993). Furthermore, Berridge and Foote (1994) also demonstrated that bilateral suppression of locus coeruleus activity substantially increases electroencephalographic measures of sedation in cortex and hippocampus. These electroencephalographic responses occur bilaterally and are only observed when locus coeruleus discharge levels are virtually completely suppressed. Accordingly, it is understandable that the inhibitory effect of 0.1-μM TAPS on the neuronal activity of locus coeruleus in the in vivo experiments may not be strong enough to cause any behavioral sedation. This explanation is also supported by the finding that when higher doses of TAPS were examined, it markedly suppressed motor activity in rats.

In summary, our data suggest that TAPS binds to μ-opioid receptors on the cell membrane of neurons of the locus coeruleus, resulting in inhibitory effects and producing hypolocomotive/sedative effects.

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