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EVIDENCE FOR DIFFERENTIAL OPIOID μ_1- AND μ_2-RECEPTOR-MEDIATED REGULATION OF HEART RATE IN THE CONSCIOUS RAT

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Summary—The possibility that μ-opioid-induced tachycardia and bradycardia could be mediated by different subtypes of the μ-receptor was studied in conscious Sprague–Dawley rats. The selective μ-receptor agonist dermorphin and its analog, TAPS (Tyr-D-Arg-Phe-sarcosine), a putative μ_1-receptor agonist, were given centrally. Tyr-D-Arg-Phe-sarcosine increased the heart rate, the response being inversely correlated to the dose (an increase of 71 ± 22, 49 ± 14 and 30 ± 17 beats/min at doses of 0.3, 3 and 30 pmol, respectively). Dermorphin induced less clear changes in heart rate (maximum increase of 39 ± 14 beats/min at the dose of 1 pmol). After treatment with the μ_1-selective antagonist naloxonazine (NAZ), TAPS 30 pmol and dermorphin 1 pmol decreased heart rate by -22 ± 10 and -24 ± 7 bpm, respectively. The bradycardic effect of larger doses of dermorphin was potentiated by NAZ (from -25 ± 8 to -97 ± 22 bpm) but abolished by the non-selective antagonist naloxone. These data suggest that the high affinity μ_1-opioid receptors mediate tachycardic responses and μ_2-receptors mediate bradycardic responses.

Key words—TAPS (Tyr-D-Arg-Phe-sarcosine), dermorphin, naloxonazine, naloxone, heart rate, blood pressure, μ-opioid receptor subtypes.

The cardiovascular effects of opiates are modified by multiple factors such as dose, route of administration, anesthesia, receptor subtype (see Feuerstein and Sirén, 1987). In the rat, central administration of μ-selective opiates induces a dose-related pressor response, which is accompanied by a biphasic heart response. At picomolar doses, selective μ-agonists such as D-Ala^2,MePhe^4,Gly-ol^5 enkephalin (DAMGO) or dermorphin produce tachycardia, whereas at larger nanomolar doses these agents produce an initial bradycardic response, followed by a sustained tachycardia (Hassen, Feuerstein and Faden, 1982; Pfeiffer, Feuerstein, Kopin and Faden, 1983a; Kiritsy-Roy, Appel, Bobbitt and van Loon, 1986; Hassen and Feuerstein, 1987; Sirén, Paakkari, Goldstein and Feuerstein, 1989). The different effects may result from multiple sites of action in the brain, since large intracerebroventricular doses may affect neuronal circuits in a wider area, more distant from the ventricular space. Indeed, different blood pressure and heart rate responses were recorded in adjacent hypothalamic nuclei (Feuerstein and Faden, 1982; Diz, Vitale and Jacobowitz, 1984). However, the opposite effects of small and large doses of μ-opiate agonists could be due to stimulation of high affinity μ_1- and low affinity μ_2-receptors (Pasternak and Wood, 1986). This assumption was prompted by recent finding that picomole doses of dermorphin, given intraventricularly, stimulated respiration by a naloxonazine (μ_1-receptor antagonist)-sensitive mechanism, while large nanomole doses of dermorphin depressed respiration, an effect which was not antagonized but potentiated by naloxonazine (Paakkari, Paakkari, Sirén and Feuerstein, 1990b; Paakkari, Paakkari and Feuerstein, 1990a).

In order to clarify which μ-receptor is involved in the central regulation of heart rate, the selective μ-agonist dermorphin (Broccardo, Erspamer, Falaconieri Erspamer, Improta, Linari, Melchiorri and Montecucchi, 1981; Rossi, de Castiglione and Perseo, 1986; Krummins, 1987) and its tetrapeptide analog, Tyr-D-Arg-Phe-sarcosine (TAPS), a putative μ_1-receptor agonist (Paakkari *et al.*, 1990a), were injected into the right lateral cerebral ventricle (i.c.v.) of conscious rats. Naloxone and the μ_1-selective antagonist naloxonazine (Hahn, Carroll-Buatti and Pasternak, 1982) were used to antagonize the effects of dermorphin and TAPS.

METHODS

Male Sprague–Dawley rats (260–340 g) were used in all experiments. After the surgical operations,

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the rats were housed individually in plastic cages (21 × 27 × 16 cm), with food and water *ad libitum*.

Surgical procedures

Rats were anesthetized with ketamine (130 mg/kg, i.m.) and acepromazine (1.3 mg/kg, i.m.). A stainless steel guide cannula was inserted stereotaxically through the skull, into the right lateral ventricle (coordinates from bregma: AP −0.8 mm, L 1.2 mm) and fixed with instant glue (Eastman 910 adhesive). On the day of the experiment, a 30 g cannula was inserted into the ventricle and the drugs were injected slowly over a period of 20 sec in a volume of 10 μl. The proper position of the intraventricular cannula was ascertained after the experiment by an injection of methylene blue (10 μl) and dissection of the brain. After the operation, the rats were allowed to recover for 2–3 days in separate cages.

For cardiovascular recording and intravenous injections, PE-50 catheters were inserted into femoral vessels under halothane anesthesia. The catheters were tunneled under the skin, exteriorized at the back of the neck and secured by a flexible spring wire, attached to the neck of the animals using an adhesive collar. The catheters were kept patent with heparinized saline (50 U/ml). The rats received an intravenous injection of naloxonazine (10 mg/kg) or saline, at the time of the surgery (24 hr before the experiment). This dose of naloxonazine and the time of pretreatment have previously been used to obtain maximum μ_1-selectivity (Hahn *et al.*, 1982, Ling, Simantov, Clark and Pasternak, 1986). The binding of naloxonazine in μ_1-receptor sites is more long-lasting than in other opioid binding sites.

Cardiovascular recording

Twenty-four hours after the surgery, the arterial line was connected to a pressure transducer (Narco Bio-Systems model RP 1500i). The mean blood pressure and heart rate were continuously recorded on a Narcotrace 80 computerized physiograph and sampled automatically every 30–60 sec by a Northstar–Hazeltine computer.

Drugs used

Dermorphin and TAPS (Peninsula, Louisville, Kentucky), naloxone (DuPont Pharmaceuticals, Wilmington, Delaware) and naloxonazine (a generous gift from Dr G. W. Pasternak, Memorial Sloan-Kettering Cancer Center, New York) were dissolved in 0.9% saline, naloxonazine with a few drops of glacial acetic acid.

Statistical analysis of the data

Data are presented as means ± SE. For comparison of two means, a t-test for independent variables was used. If the one way analysis of variance (ANOVA) indicated statistical difference between the groups observed at a given time, the analysis was proceeded by the multiple comparison test of Newman–Keul. The impact of changes within a group along the time axes, as well as the overall statistical differences between groups, were studied by means of 2-way ANOVA for repeated measures.

The area under the curve (AUC) was calculated according to the trapezoidal method. In brief, if the two consecutive points of the x-axis (time) are denoted $x1$ and $x2$ and the corresponding y-values $y1$ and $y2$, respectively, the area (A) under such a fragment of the curve is expressed by the equation $A = (x2 - x1)y2 - (y2 - y1)(x2 - x1)/2$. Accordingly, the AUC equals to the sum of the areas corresponding each time interval.

RESULTS

Effect of TAPS on heart rate and blood pressure

At 0.3, 3 and 30 pmol, TAPS increased the heart rate (Fig. 1A). The magnitude of the tachycardic response was inversely correlated to the dose. The

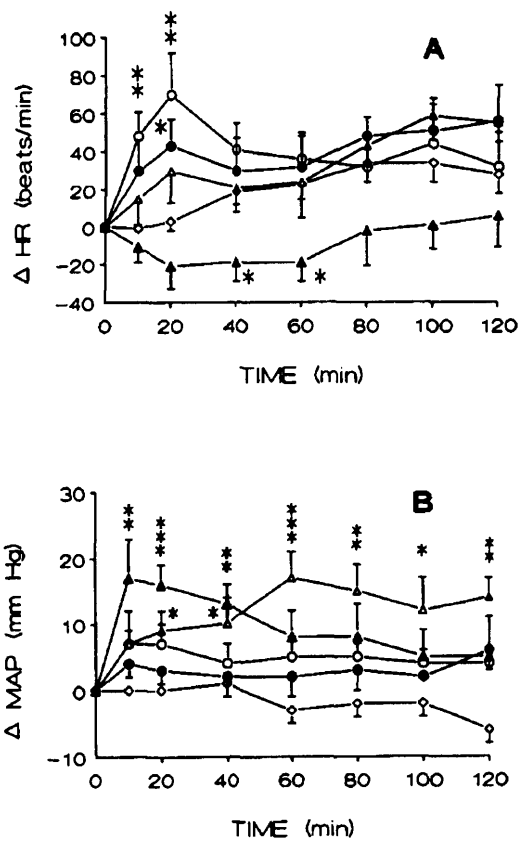

Fig. 1. Effect of intraventricular administration of Tyr-D-Arg^2-Phe-(NMe)Gly^4(TAPS) on heart rate (HR, A) and mean arterial pressure (MAP, B) in the conscious rat. Increasing doses of TAPS were injected intraventricularly. Vertical bars indicate SE. Number of rats = 6 in each group. (○) TAPS 0.3 pmol, (●) TAPS 3 pmol, (△) TAPS 30 pmol, (▲) TAPS 300 pmol, (◇) NaCl. *$P < 0.05$, **$P < 0.01$ Statistical difference between TAPS-treatment vs control (Newman–Keul test). Two way ANOVA for repeated measures: time $P < 0.05$ (HR), $P = 0.18$ (MAP); group $P < 0.05$ (HR), $P < 0.01$ (MAP).

largest dose (300 pmol) induced a slight bradycardia (maximally -20 ± 12). The doses of 30 and 300 pmol increased blood pressure significantly (Fig. 1B).

The initial levels of heart rate (beats/min), before administration of TAPS, were 358 ± 7 (0.3 pmol), 390 ± 10 (3 pmol), 362 ± 9 (30 pmol) and 367 ± 6 (300 pmol). The corresponding values for mean arterial pressure (mmHg) were 110 ± 3, 124 ± 4, 115 ± 5 and 106 ± 6. The differences between the groups were not statistically significant.

Effect of dermorphin on heart rate and blood pressure

Dermorphin, at 1 pmol, slightly increased the heart rate. The maximum effect, 39 ± 14 beats/min ($P < 0.05$), was obtained 50 min after the injection. At 10 pmol, dermorphin had no significant effect on the heart rate (Fig. 2). Neither of the doses changed the blood pressure significantly; maximum changes from the baseline were in mmHg 4 ± 5 (1 pmol) and 6 ± 5 (10 pmol), during the 120 min period of registration.

Effect of naloxonazine on cardiovascular actions of dermorphin and TAPS

Pretreatment with naloxonazine (10 mg/kg, i.v., 24 hr before the experiment) abolished the tachycardic and hypertensive effect of TAPS (Figs 3A and B).

After pretreatment with naloxonazine, dermorphin 1 and 10 pmol induced bradycardia (Fig. 2), which was statistically significant (one-way ANOVA for repeated measures) during the first 30 min (1 pmol; $P < 0.02$) and 40 min (10 pmol; $P < 0.05$). To study the effect of naloxonazine on profound bradycardia, one group of rats was given a large dose of 20 nmol dermorphin. After pretreatment with naloxonazine the heart rate decreased significantly more than in control group, whereas the blood pressure response was not changed by naloxonazine (Fig. 4).

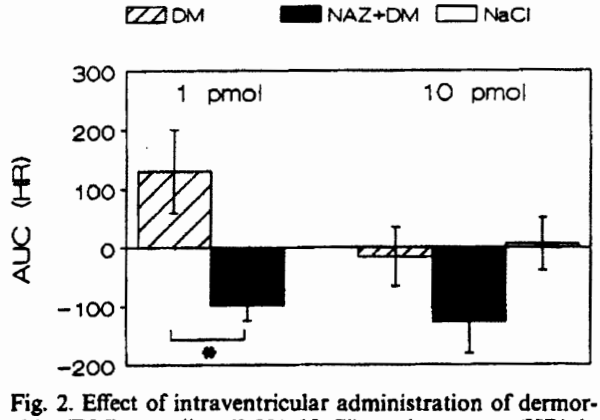

Fig. 2. Effect of intraventricular administration of dermorphin (DM) or saline (0.9% NaCl) on heart rate (HR) in vehicle- and naloxonazine (NAZ, 10 mg/kg, i.v., 24 hr before DM)-treated conscious rats. Vertical bars indicate SE. Number of rats = 6 in each group. AUC = area under the time–response curve (see Methods). Asterisk indicates significant difference between saline and NAZ-treated group (*t*-test for independent observations); $*P < 0.05$.

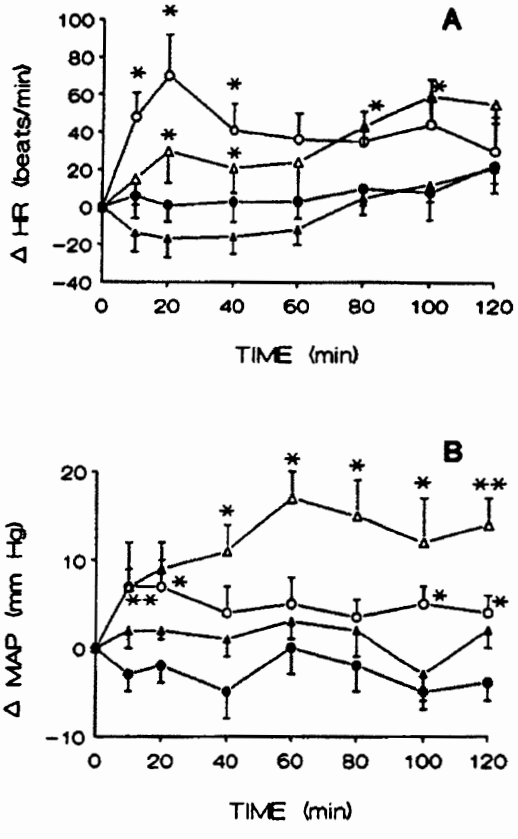

Fig. 3. Effect of naloxonazine (NAZ, 10 mg/kg, i.v., 24 hr before) on the cardiovascular actions of Tyr-D-Arg^2-Phe-(*N*Me)Gly^4(TAPS), given intraventricularly in the conscious rat. Vertical bars indicate SE. Number of rats = 6 in each group. (A) heart rate (HR), (B) mean arterial pressure (MAP). The base level of heart rate in each group was 358 ± 7 (TAPS 0.3 pmol), 347 ± 4 (TAPS 30 pmol), 391 ± 10 (NAZ + TAPS 0.3 pmol) and 411 ± 13 beats/min (NAZ + TAPS 30 pmol). The corresponding values for blood pressure were 110 ± 3 mmHg, 113 ± 4 mmHg, 121 ± 4 mmHg and 115 ± 3 mmHg, respectively. (○) TAPS 0.3 pmol, (△) TAPS 30 pmol, (●) NAZ + TAPS 0.3 pmol, (▲) NAZ + TAPS 30 pmol. Asterisks denote statistical significance between saline- and NAZ-treated groups (*t*-test for independent observations); $*P < 0.05$, $**P < 0.01$. Two way ANOVA for repeated measures: time $P < 0.07$ (HR), $P = 0.05$ (MAP); group $P < 0.01$ (HR), $P < 0.01$ (MAP).

Effect of naloxone on cardiovascular actions of dermorphin

Pretreatment of rats with naloxone (5 mg/kg, i.v., 20 min before dermorphin), completely antagonized the bradycardic and hypertensive effect of 300 pmol of dermorphin (Fig. 5). Naloxone did not change heart rate or blood pressure significantly.

DISCUSSION

There is evidence for two subtypes of opioid μ receptors, each having distinct actions (see Pasternak, 1988; Blurton, Broadhurst, Wood and Wylie, 1986; Pasternak and Wood, 1986). Based on morphological studies using the relatively selective μ_1-antagonist, naloxonazine (Hahn *et al.*, 1982) the μ_1-receptors have been implied to mediate supraspinal analgesia

and μ_2-receptors respiratory depression (Ling, Spiegel, Lockhart and Pasternak, 1985; Bodnar, Williams, Lee and Pasternak, 1988). Studies with naloxonazine further suggested that the bradycardic effect of opioids would be mediated by μ_2-receptors (Holaday, Pasternak and Faden, 1983). In the present study, the cardiovascular effects of dermorphin and its tetrapeptide analog, TAPS were examined to find out whether μ_1- and μ_2-mediated responses could be differentiated.

The biphasic effect of μ-agonists on heart rate described here, tachycardia at small doses, bradycardia at large doses, is in accordance with previous results on intracerebroventricular administration of opioids (Pfeiffer, Feuerstein, Zerbe, Faden and Kopin, 1983; Sirén *et al.*, 1989). Local injections of μ-selective opioids, such as DAMGO or dermorphin into the brainstem or forebrain cardiovascular nuclei (nucleus tractus solitarius, nucleus ambiguus, preoptic and anterior hypothalamic nuclei), also induced tachycardia at small doses and bradycardia at larger doses (Hassen *et al.*, 1982; Hassen, Feuerstein and Faden, 1984; Diz *et al.*, 1984; Pfeiffer *et al.*, 1983a; Kiritsy-Roy *et al.*, 1986; Sirén and Feuerstein 1991). Zhu and Szeto (1989) reported that in fetal lambs, morphine-infusion produced a dose-dependent tachycardia, with no change in blood pressure but at larger doses (more than 2.5 mg/hr) the response was reduced, resulting in a bell-shaped dose–response curve.

Also (D-Ala^2,D-leu^5)enkephalin (DADL), a δ-agonist which binds to μ_1-receptors as well (Lutz, Cruciani, Munson and Rodbard, 1985), increased the heart rate when small doses (0.2–7.5 nmol) were injected into anterior hypothalamic and septal areas of the brain but the μ-selective DAMGO was approximately 10-fold more potent than DADL (Pfeiffer *et al.*, 1983a). Chronic treatment of rats

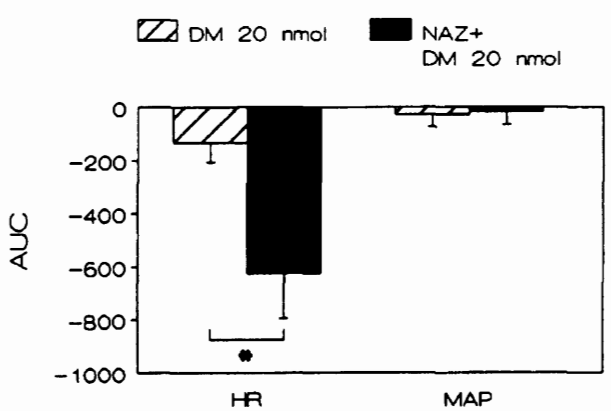

Fig. 4. Effect of naloxonazine (NAZ, 10 mg/kg, i.v., 24 hr before) on the cardiovascular responses to a large dose (20 nmol) of dermorphin (DM), given intraventricularly, in the conscious rat. Vertical bars indicate SE. Number of rats = 6 in each group. The baseline heart rates were 423 ± 9 beats/min in the DM 7 nmol group and 394 ± 15 beats/min in the MAZ + DM 7 nmol group. Asterisk indicates significant differences between saline- and NAZ-treated groups (*t*-test for independent observations).

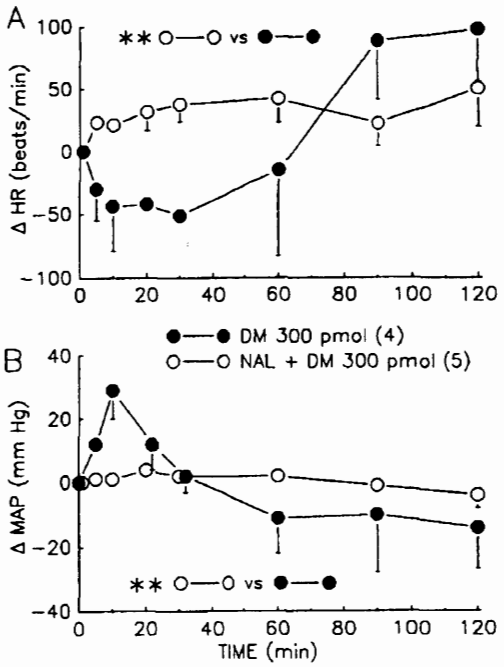

Fig. 5. Effect of naloxone (NAL, 5 mg/kg, i.v., 20 min before) on the cardiovascular actions of dermorphin (DM 300 pmol), given intraventricularly in the conscious rat. (A) shows the changes in heart rate (HR), (B) illustrates the corresponding changes in mean arterial pressure (MAP). The baseline level of heart rate was 398 ± 17 in the DN 300 pmol group and 364 ± 17 beats/min in NAL + DM 1 nmol group. The corresponding levels of blood pressure were 112 ± 1 and 115 ± 1 mmHg, respectively. (●) DM, (○) NAL + DM. $^{**}P < 0.05$ (*t*-test for independent observations).

with naloxone, which increased binding of μ-opioid receptors (DAMGO) by 180% in the brain stem and by 80% in the anterior hypothalamic area, abolished the tachycardiac and pressor responses to systemic administration of morphine but resulted in supersensitivity to its depressor and bradycardic effects (Pfeiffer, Pfeiffer, Feuerstein, Faden and Kopin, 1984). These findings concur with the differential distribution of μ_1 vs μ_2-binding sites in the brain of the rat. μ_1-Binding sites represent about 60% of total μ-opioid binding in the hypothalamus, as compared to the 15% of μ_1-sites in the hindbrain (Goodman and Pasternak, 1985). Stimulation of μ-opioid receptors produces tachycardia at small doses in both forebrain and brainstem nuclei, however. In a continuous intravenous infusion model, tolerance developed rapidly to analgesia (a μ_1-effect), whereas tolerance to respiratory depression and gastrointestinal transit (μ_2-effects), developed at a much slower rate (Ling, Simantov and Pasternak, 1987). Accordingly, rapid tachyphylaxis to the μ_1-induced tachycardia but not to the μ_2-mediated bradycardia, might contribute also to the bell-shaped dose–response relationship of μ-opioids on heart rate.

Recently it was described that the highly μ-selective opioid peptide, dermorphin, at picomolar

doses induced respiratory and locomotor stimulation, whereas at larger doses it depressed respiration (Paakkari *et al.*, 1990b). The selective μ_1-receptor antagonist, naloxonazine (Hahn *et al.*, 1982), blocked the stimulatory effects but potentiated the respiratory depression. It was suggested that, at very small doses, dermorphin binds to high-affinity, respiratory stimulating μ_1-sites only, whereas at larger doses, the μ_2-receptor-mediated depressant effects dominate.

In the present study, dermorphin at a small dose of 1 pmol produced a slight tachycardia, whereas the larger doses decreased the heart rate, as reported earlier (Sirén *et al.*, 1989). Tyr-D-Arg-Phe-sarcosine, which is at least 3-fold more potent than the parent heptapeptide in analgesia tests (Sato, Sakurada, Sakurada, Furuta, Chaki, Kisara, Sasaki and Suzuki, 1987), produced a more profound tachycardia, at the doses of 0.3–30 pmol. A dose of 300 pmol, which induced maximum analgesia and catalepsy, that lasted at least 2 hr, induced a negligible decrease in the heart rate. Tyr-D-Arg-Phe-sarcosine does not depress but stimulates respiration even at large doses, which suggests that it may have more agonist activity on μ_1- than μ_2-receptors (Paakkari *et al.*, 1990a). Preliminary results of binding studies from this laboratory support this concept (S. Vonhof, personal communication). Accordingly, it is assumed that the tachycardic responses might be μ_1-receptor-mediated effects.

The pressor responses to TAPS were not clearly dose-related at the small doses, while the largest dose of 300 pmol induced a significant increase in mean arterial pressure. The tachycardic response to TAPS, to the contrary, was inversely related to the dose. Thus, it may be possible that these hemodynamic effects are mediated by different mechanisms. However, both the pressor and tachycardic effects of TAPS seemed to involve an activation of a μ_1-site as they were effectively blocked by naloxonazine. Moreover, the inverse relationship of the TAPS-induced heart rate and blood pressure responses could be due to reflex bradycardia masking the tachycardic effect of larger doses of TAPS. Surprisingly, the pressor responses after large nanomole doses of dermorphin seemed not to be due to activation of μ_1-receptors since naloxonazine did not block these responses, although they were effectively blocked by the unselective opioid antagonist naloxone. One interpretation is that the large dose (20 nmol) of dermorphin might bind to δ-opioid receptors, which have been suggested to mediate pressor responses (Holaday, 1983).

The hypothesis that μ_1-receptors mediate tachycardia was tested by pretreating the rats with naloxonazine. A relative selectivity of naloxonazine for μ_1-sites is reportedly obtained with the dose of 10–20 mg/kg (rat) and about 24 hr after the administration; the short-lasting binding or binding at larger doses is less selective (Hahn *et al.*, 1982, Johnson and Pasternak, 1984; Ling *et al.*, 1986).

Naloxonazine prevented the dermorphin- or TAPS-induced tachycardia. Actually, the μ_1-blockade reversed the slight tachycardia produced by a very small dose of dermorphin (1 pmol) into bradycardia. After treatment with naloxonazine, the bradycardic effect of a large dose of dermorphin was potentiated, evidently because of the absence of the stimulatory μ_1-effect. The effect was specific to pretreatment with naloxonazine, since the non-selective opiate antagonist, naloxone, abolished both the tachycardiac and bradycardic responses to dermorphin. δ-Receptors may mediate at least a part of the bradycardia induced by the large dose (20 nmol) of dermorphin but due to the high μ-selectivity of dermorphin, it is implausible that at small doses it would induce other than μ-receptor-related effects.

The results suggest that μ_1-receptors mediate the positive chronotropic effect and μ_2-receptors the negative chronotropic effect of selective μ-agonists. Another interpretation is that central μ_1-receptors might stimulate sympathetic outflow to the heart and μ_2-receptors to the vasculature, a hypothesis which should be investigated in the future in animals with baroreflex deafferentation.

In summary, the present data demonstrated that, unlike dermorphin, TAPS at the doses of 0.3 and 30 pmol did not produce significant bradycardia after naloxonazine. Furthermore, TAPS induced more tachycardia and less bradycardia than dermorphin, indicating that it may act predominantly as a μ_1-agonist, as suggested previously.

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