

Body Temperature and Analgesic Effects of Selective μ and κ Opioid Receptor Agonists Microdialyzed into Rat Brain¹

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ABSTRACT

Opioids administered by i.c.v. injection produce body temperature (T_b) changes and analgesic responses in rats. The present study was undertaken to investigate the effects on T_b and analgesia of highly selective μ and κ opioid receptor agonists and antagonists delivered directly into the preoptic anterior hypothalamus (POAH) and periaqueductal gray (PAG) by the intracerebral microdialysis method. Microdialyzed into the POAH, the μ receptor agonist Tyr-Pro-N-MePhe-D-Pro-NH₂ induced dose-related hyperthermia that could be prevented or antagonized by the μ receptor antagonist cyclic D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH₂ or by naloxone, but not by the κ receptor antagonist nor-binaltorphimine. The κ receptor agonist dynorphin A₁₋₁₇, microdialyzed into

the POAH, induced dose-related hypothermia that was prevented or antagonized by nor-binaltorphimine but not cyclic D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH₂. Neither Tyr-Pro-N-MePhe-D-Pro-NH₂ nor dynorphin A₁₋₁₇ microdialyzed into the PAG produced significant changes in T_b . However, these agonists microdialyzed into the PAG produced analgesic responses that did not occur after administration into the POAH. These results support the hypothesis that the hyperthermic response to opioids is mediated by the μ receptor and the hypothermic response is mediated by the κ receptor in rats. The POAH is a primary functional area in T_b , but not in analgesic, responses to opioids, whereas the PAG is a sensitive area for analgesic, but not for T_b , responses to opioids.

Opioid drugs and opioid peptides alter T_b (Lotti *et al.*, 1966, Clark and Lipton, 1985). The direction and magnitude of T_b responses caused by opioids vary widely under different test conditions. Although the detailed mechanisms of these variations are still not known completely, there is little doubt that opioid receptors are involved in thermoregulation (Geller *et al.*, 1983; Adler *et al.*, 1988; Burks, 1991; Adler and Geller, 1993).

Highly selective agonists and antagonists can be used to distinguish the specific action on T_b mediated through one type of opioid receptor from the others in the brain. Previous results from this and other laboratories demonstrated that i.c.v. administration of selective μ receptor agonists produced hyperthermia (Adler and Geller, 1993; Spencer *et al.*, 1988; Handler *et al.*, 1992) that could be blocked or antagonized by selective μ receptor antagonists, whereas κ receptor agonists produced hypothermia (Spencer *et al.*,

1988; Adler *et al.*, 1983; Adler *et al.*, 1986) that could be blocked or antagonized by selective κ receptor antagonists (Cavicchini *et al.*, 1988; Handler *et al.*, 1992). On the basis of findings such as these, we hypothesized that the hyperthermic response to opioids is mediated by the μ receptor and the hypothermic response is mediated by the κ receptor (Geller *et al.*, 1982; Geller *et al.*, 1986; Adler *et al.*, 1988). However, it was not known whether the same effects would occur when those agonists or antagonists were administered directly into the POAH, a vital region in T_b regulation, rather than by the i.c.v. route, which allows the drugs to diffuse rapidly throughout the brain.

The POAH is generally considered to be the primary site for central control of T_b , because a large population of thermosensitive neurons is located there (Boulant, 1980) and because its destruction or inactivation disrupts thermoregulation. It receives and integrates the T_b information from both central and peripheral sensors and sends the modulating signal to direct T_b -regulatory effectors for maintaining T_b around a given temperature, the set point. It has been suggested that the POAH is the site of action of opioids that are given centrally and have effects on T_b (Lotti *et al.*, 1966; Tseng *et al.*, 1980; Stanton *et al.*, 1985). At least three types of opioid receptors, μ , κ and δ , have been discovered so far within the POAH (Man-

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ABBREVIATIONS: aCSF, artificial cerebrospinal fluid; CTAP, cyclic D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH₂; Dyn, dynorphin A₁₋₁₇; MPA, maximum possible analgesia; nor-BNI, nor-binaltorphimine; PAG, periaqueductal gray; PL017, Tyr-Pro-N-MePhe-D-Pro-NH₂; POAH, preoptic anterior hypothalamus; T_b , body temperature.

sour *et al.*, 1987), and opioids alter the activities of the thermosensitive neurons within this region (Baldino *et al.*, 1980; Lin *et al.*, 1984).

The PAG is known to be one of the most important regions involved in pain modulation (Basbaum and Fields, 1984). It has also been reported to be involved in opioid-induced T_b responses (Tseng *et al.*, 1980). Previous results from this laboratory have demonstrated the analgesic effects of selective opioid receptor agonists, given i.c.v., using the cold-water tail-flick test (Pizziketti *et al.*, 1985; Tiseo *et al.*, 1988; Adams *et al.*, 1993), but it was not known whether these agonists, microdialyzed into the PAG, would also affect T_b .

The intracerebral microdialysis method provides a new approach either to delivery of drugs into, or to extracellular collection of neurochemicals from, a selected brain area (Ungerstedt, 1991). Microdialysis of a substance obviates contact between fluid and tissue and therefore minimizes the local irritation inherent in most other intracerebral injection procedures. Thus the method may mimic closely the release of a substance under physiological conditions (Westerink and Justice, 1991). Furthermore, drug delivery by microdialysis can be conducted in conscious, freely moving animals without handling the animal, thus avoiding physical restraint and stress that can affect T_b and analgesic responses.

In the present study, we investigated the effects of the activation of μ and κ opioid receptors on both T_b and analgesia by using highly selective μ and κ opioid receptor agonists and antagonists microdialyzed directly into the POAH or the PAG of rats. All drugs were administered to freely moving animals to avoid effects of anesthesia or restraint on T_b and analgesia.

Materials and Methods

Animals

Male Sprague-Dawley rats (Zivic-Miller, Pittsburgh, PA) weighing 250 to 300 g were used in this study. They were housed 3 to 4 per

cage for at least 1 week before experimental use and were fed laboratory chow and tap water *ad libitum*. The temperature of the animal room was $22 \pm 2^\circ\text{C}$, and a 12-hr light/12-hr dark cycle was used.

Microdialysis Probes

The microdialysis probes used in this study were constructed as follows: Cellulose fibers (Spectrum Medical Industries, Los Angeles, CA; M W cutoff 6 kDa and I.D. = $150 \mu\text{m}$) were used as microdialysis tubing for the perfusion probe. The probes consisted of two parallel, soldered stainless steel, 25-gauge cannulas with a U-shaped loop of microdialysis tubing 2 mm in effective length at their tips. The two parts were joined by epoxy. A fine (0.075-mm) tungsten wire (World Precision Instruments, Inc. Sarasota, FL) was preincorporated into the loop to provide the necessary stiffness and to prevent the open ends of the loop from closing. The remaining two open ends of the cannulas were connected to PE-20 tubing as input and output cannulas, respectively (fig. 1A).

Surgery and Probe Implantation

Eight days before the experiment, each rat was anesthetized with an i.p. injection of a mixture of ketamine hydrochloride (100–150 mg/kg) and acepromazine maleate (0.2 mg/kg), and a 20-mm, 17-gauge stainless steel guide cannula with an indwelling stylet was stereotaxically implanted unilaterally into the POAH (AP: 7.8, R: 1.0, V: -1) or PAG (AP: 0.6, R: 0.8, V:1) (Pellegrino and Cushman, 1967). The guide cannula was fixed with dental cement and self-tapping bone screws. After surgery, the animals were housed individually to prevent them from destroying the cannulas. One week later, the animals were anesthetized again with ketamine, and the stylet was replaced by a microdialysis probe such that its dialysis membrane tip protruded exactly 1 mm beyond the guide. It, too, was fixed to the skull with dental cement. The open ends of the probe were protected by two short pieces of PE-20 tubing that were sealed on their top ends. In order to avoid the influence on T_b of acute injury produced by insertion of the probe, the animals were allowed to recover for another 24 hr before the experiments were begun.

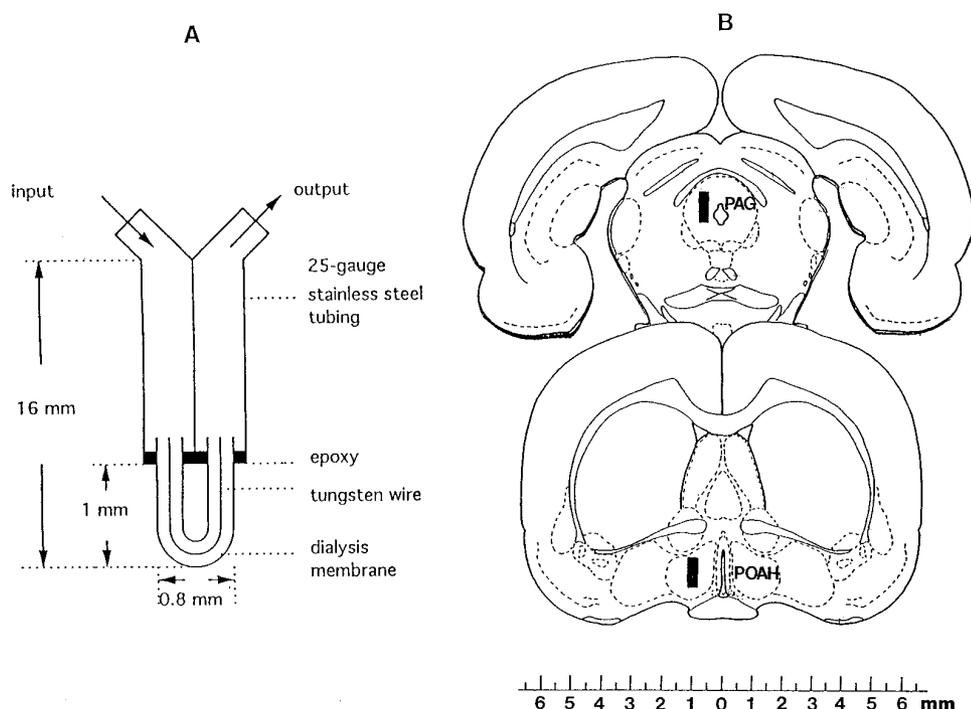


Fig. 1. A) Schematic drawing of a microdialysis probe used in these experiments. B) Coronal section of the rat brain, showing the location of the microdialysis probe (shaded region: dialysis membrane).

Drugs

The following drugs were tested: the *mu* receptor agonist PL017; the *mu* receptor antagonist CTAP; the *kappa* receptor agonist Dyn (Multiple Peptide Systems, San Diego, CA), the *kappa* receptor antagonist nor-BNI (Research Biochemicals International, Natick, MA) and the general opioid antagonist, naloxone (National Institute on Drug Abuse, Rockville, MD). Drug doses were chosen on the basis of prior i.c.v. experiments in which a dose-response curve was obtained. In most cases, aCSF (composition in mM: NaCl, 125; KCl, 2.5; NaHCO₃, 27; NaH₂PO₄, 0.5; Na₂HPO₄, 1.2; CaCl₂, 1.2; MgCl₂, 1; ascorbic acid, 0.1; glucose, 5; pH 7.4) was the vehicle and control solution. For purposes of comparison, sterile pyrogen-free isotonic 0.9% saline was also used as a vehicle or control in some cases.

Experimental Protocols

Evaluation of the dialysis probe. Both *in vitro* and *in vivo* studies were conducted to assess the efficiency of the dialysis probes in delivering the opioid receptor agonists and antagonists. During the *in vitro* studies, probes were tested by perfusing the agonists or antagonists through them at a rate of 1 μ l/min while their tips were immersed in microtubes containing 60 μ l saline at 37°C. The 10- μ l samples were taken from the microtubes after 15, 30, 60, 120 and 180 min of perfusion. During the first 60-min period, 5- μ l samples were also taken from the outflow collections. The concentration of the drugs in each sample and in perfusion vehicles was measured by HPLC. We calculated what percentage of the drug concentration in the dialysis perfusate was represented by the percentage (*Y*) of the drug concentration in the bathing medium by dividing the concentration per microliter of the medium solution (*C_m*) by that of the input solution (*C_i*):

$$Y = C_m / C_i \times 100$$

To determine the amount of drug that may have been lost (or that stuck to the membrane or tubing) during the 60-min perfusion period, we calculated the difference (*A_{lost}*) between drug amount in the input solution and that in the output solution as follows:

$$A_{lost} = [C_i - (C_o + C_m)] \times 60 \mu\text{l}$$

where *C_o* is the output concentration. During *in vivo* studies, the drugs were perfused through the probes *in situ* in the POAH. The effluents were collected over intervals of 0 to 0.5, 0.5 to 1, 1 to 2 and 2 to 3 hr during perfusions. The rates (*Z*) of drugs perfused into the dialysis system were estimated by the concentration differences between the output and input solutions:

$$Z = [1 - (C_o/C_i)]fC_i$$

where *C_i* and *C_o* are the average input and output concentrations of the corresponding collecting intervals, respectively, and *f* is the flow rate of the perfusion, 1 μ l/min; *C_o/C_i* was estimated by dividing the drug concentration of the output solution by that of the input solution. The amount (*A*) of drug actually delivered into the brain during the first 60-min perfusion period was estimated as follows:

$$A = [(Z_{30} \times 30 \text{ min}) + (Z_{60} \times 30 \text{ min})] - A_{lost}$$

Microdialysis experiments. Experiments were performed between 8:30 A.M. and 4:00 P.M. On the test day, the rats were placed into individual plastic cages in an environmental room kept at 21°C \pm 0.3°C and 52% \pm 2% relative humidity. At the beginning of the experiment, the sealed tubing on both input and output cannulas of the microdialysis probes was removed, and the input cannulas were connected by PE-20 tubing to a single-channel swivel (series 375, Instech Laboratories, Inc., Plymouth Meeting, PA) that was then connected to a 1-ml tuberculin syringe (Becton Dickinson & Co., Rutherford, NJ) clamped to a perfusion pump (Harvard Apparatus, Inc., South Natick, MA). *T_b* measurements were made according to standard procedures in our laboratory. After a 1-hr acclimation

period, a thermistor probe (YSI series 400, Yellow Springs Instrument Co., Inc., Yellow Springs, OH) was lubricated and inserted approximately 7 cm into the rectum; *T_b* measurements were read from a digital thermometer (Model 49 TA, YSI). During the readings, the rat's tail was held gently between two fingers, and the animal was otherwise free to move about. The first three measurements were taken at 30-min intervals. To allow for adaptation to the procedure, the first reading was discarded, and the next two were averaged to establish a base line. In this way, each animal served as its own control. Experimental values were then compared to the predrug base-line values obtained for each animal. Immediately after the third measurement, the drug of interest was perfused through the microdialysis probe at the rate of 1 μ l/min over a 1-hr period or, in some cases, a 3-hr period. As soon as drug perfusion was ended, sterile pyrogen-free saline was substituted for the drug and perfused at a rate of 8 μ l/min for 1 min, or, in some cases, a second drug was perfused immediately after the first drug perfusion ended.

Analgesia testing. The cold-water tail-flick test was used to assess the analgesic effects of opioid receptor agonists according to standard procedures in our laboratory (Pizziketti *et al.*, 1985). A 1:1 mix of ethylene glycol/water was maintained at -3°C with a circulating water bath (Model 9500, Fisher Scientific, Pittsburgh, PA). Animals were held firmly over the opening of the bath, and their tails were submerged approximately halfway into the solution. The nociceptive threshold was taken as the latency until the rat removed or flicked its tail. Three predrug latencies were measured: 60, 30 and 0 min before drug perfusion. For each animal, the first reading was discarded to minimize variability, and the remaining two were averaged to determine the base-line latency. After 60 min of drug perfusion, latency to tail-flick was tested at 15, 30, 60 and 120 min. If an animal did not respond within 60 sec, the trial was terminated, and a maximum latency of 60 sec was recorded. The analgesic effect of drug treatment was calculated for each rat as follows:

$$\% \text{MPA} = [(\text{postdrug latency} - \text{base-line latency}) / (60 - \text{base-line latency})] \times 100.$$

HPLC procedures. The samples taken from both *in vitro* and *in vivo* probe evaluation tests were analyzed by reverse-phase HPLC (Model 1050, Hewlett Packard, Co., San Fernando, CA). The analysis was performed at room temperature with a C₄ column (5 μ m, 4.6 \times 25 mm, Vydac), a 1050 quaternary pump, a 1050 dual-wavelength detector and two integrators (all from Hewlett Packard). The mobile phase consists of two solutions: 0.1% trifluoroacetic acid in water and 0.1% trifluoroacetic acid in 80% acetonitrile. A linear gradient of acetonitrile (0%–50%) at 1 ml/min is used, followed by isocratic elution with acetonitrile (50%). Drug standards or samples were dissolved in a small volume of 0.1% trifluoroacetic acid, filtered through a 0.22- μ m filter (Milipore) and injected onto a C₄ column.

Verification of dialysis probe placement. At the conclusion of the experiments, animals were placed into a bucket containing dry ice within a metal mesh basket for at least 10 min. The animals were almost instantaneously anesthetized by the carbon dioxide, rapidly asphyxiated and cooled. Their brains were excised and coronally cut, and the visible track of the microdialysis probe was checked. In some cases, bromophenol blue (0.2%) was microdialyzed into the POAH for 3 hr or into the PAG for 1 hr, and the brain was removed and frozen. A block of tissue containing the track of the probe and the stain of the dialyzed dye was cut and checked (fig. 2). Data from rats in which the probes were not located within the POAH or PAG (approximately 10%) were not included in the results.

Statistical Analysis

The results are reported as *T_b* changes (ΔT , means \pm S.E.) from base line and % MPA (means \pm S.E.). These changes were analyzed for each agonist or agonist/antagonist combination using a one-way analysis of variance with a repeated-measure variable of time, fol-

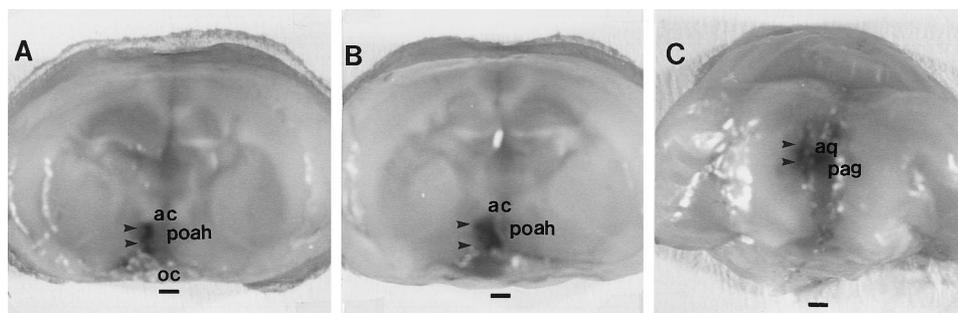


Fig. 2. Photographs of coronal sections of rat's brain showing spread of bromophenol blue after 1 hr (panel A) or 3 hr (panel B) of dialysis into the POAH and after 1 hr of dialysis into the PAG (C). Arrows point to edge of dye stain. Horizontal bar = 1 mm in length. ac: anterior commissure; aq: aqueductus; oc: optic chiasm; pag: periaqueductal gray; poah: preoptic anterior hypothalamus.

lowed by a *post-hoc* Fisher's test. Treatment differences at each time-point were compared with aCSF controls by using the modified *t* statistic, in which the significance level is reduced by the Bonferroni procedure (Wallerstein *et al.*, 1980). The 5% level of probability was accepted as statistically significant.

Results

The range of drug diffusion within the POAH and PAG. The range of drug diffusion within the brain regions is estimated by the stain of the bromophenol blue dialyzed into the POAH (1-hr and 3-hr perfusion) and the PAG (1-hr perfusion). The results are illustrated in figure 2. After a 1-hr perfusion, the dye diffused around the probe tip and created a spherical stain 1.2 mm in diameter within the POAH (fig. 2A) and the PAG (fig. 2C). The spread of the dye increased to 1.6 mm in diameter after a 3-hr perfusion into the POAH, but it still did not extend outside of the POAH (fig. 2B).

The efficiency of the dialysis probes in delivering drugs. The amounts of PL017, Dyn, naloxone, CTAP and nor-BNI that crossed the dialysis membrane and diffused into the medium *in vitro* are shown in figure 3. There was some absorption of Dyn and CTAP by the dialysis probe or perfusion/collection tubings, because 18% and 14% of the input amount for these two drugs, respectively, was lost during the 60-min perfusion (table 1). *In vivo*, PL017 diffused into the dialysis system at a rate of 0.14 ± 0.006 nmol/min during the first 30-min perfusion period and slowed to a rate of 0.12 ± 0.004 nmol/min during the 120 to 180-min perfusion period (fig. 4, top). Dyn diffused into the system at a rate of 0.074 ± 0.013 nmol/min during the first 30-min perfusion period and at a rate of 0.065 ± 0.018 nmol/min during the

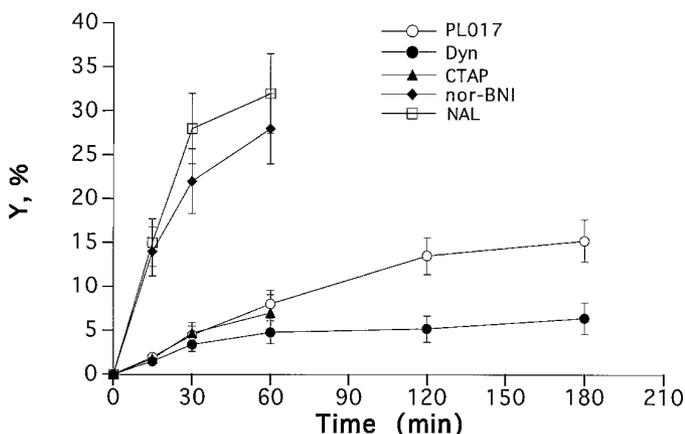


Fig. 3. Time course of the diffusion of drugs across the dialysis membrane to surrounding medium *in vitro*. Y: ratio (expressed as percentage) of concentration of drug in medium to that in dialysis tube.

TABLE 1

Estimation of the amount of drugs (nmol) perfused into the dialysis system and the amount that actually passed into the brain during 60 min of *in vivo* perfusion ($n = 6$)

Drugs (input)	Amount into the System ^a ($Z \times \text{time}$)	Amount Lost (A_{lost})	Amount into the Brain ^b (A)
PL017 (1.69/ μl)	8.1 8%	1.32 1.3%	6.78 6.7%
Dyn (0.35/ μl)	4.65 22%	3.9 18.7%	0.69 3.3%
CTAP (0.9/ μl)	11 20%	7.6 14%	3.2 6%
nor-BNI (1.2/ μl)	24.4 33%	2.9 4%	21.5 29%
NAL (1.3/ μl)	27 34%	2.4 3%	24.6 31%

^a Amount of drug perfused into the system was calculated by $(Z_{30} \times 30 \text{ min}) + (Z_{60} \times 30 \text{ min})$ based on *in vivo* test (see "Materials and Methods" for details). The percentage was the ratio of the drug amount to that in the input solution.

^b Amount of drug delivered into the brain was calculated by $A = [(Z_{30} \times 30 \text{ min}) + (Z_{60} \times 30 \text{ min})] - A_{\text{lost}}$, where A_{lost} was determined by *in vitro* test (see "Materials and Methods" for details).

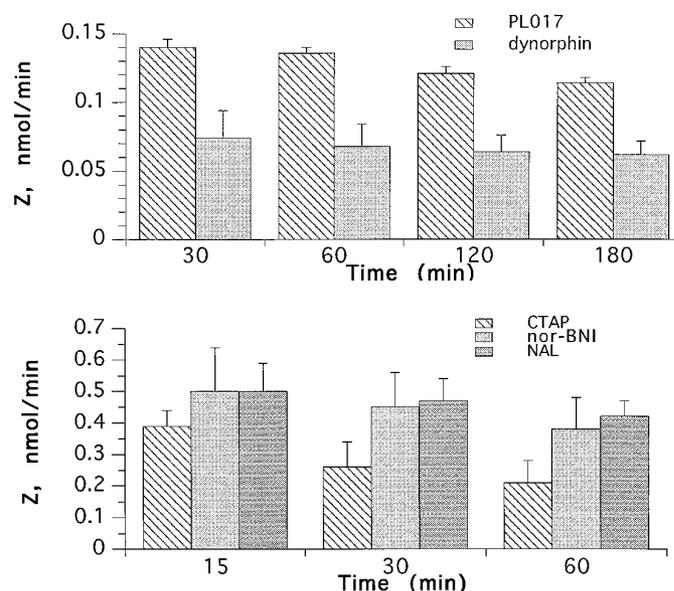


Fig. 4. Top panel) Rates of PL017 (1.69 nmol/ μl) and Dyn (0.35 nmol/ μl) diffusion into brain tissue *in vivo* during time intervals 0 to 30, 30 to 60, 60 to 120 and 120 to 180 min of dialysis. Bottom panel) Rates of CTAP (0.9 nmol/ μl), nor-BNI (1.2 nmol/ μl) and naloxone (1.3 nmol/ μl) diffusion into brain tissue *in vivo* during time intervals 0 to 15, 15 to 30 and 30 to 60 min of dialysis.

120 to 180-min perfusion period (fig. 4, top). The diffusion rates of CTAP, nor-BNI and naloxone into the dialysis system are shown in the bottom panel of figure 4. On the basis

of the percentage of drug lost as observed in *in vitro* tests, the amounts of drugs that passed through the dialysis membrane and were actually delivered to the brain during *in vivo* tests are estimated as shown in table 1.

T_b responses to the *mu* receptor agonist PL017 microdialyzed into the POAH. A 3-hr perfusion of vehicle (saline or aCSF) into the POAH produced no change in T_b (fig. 5, top). However, 3 hr of perfusion of PL017 induced a dose-related hyperthermia (fig. 5, bottom). The maximum T_b changes, which occurred about 180 min after the perfusions began, were $1.1 \pm 0.13^\circ\text{C}$, $1.72 \pm 0.12^\circ\text{C}$ and $2.0 \pm 0.18^\circ\text{C}$ for doses of $0.32 \text{ nmol}/\mu\text{l}$, $1.69 \text{ nmol}/\mu\text{l}$ and $3.38 \text{ nmol}/\mu\text{l}$, respectively. The recovery time course in which T_b returned to base line was 180 min for the lowest dose ($0.32 \text{ nmol}/\mu\text{l}$), whereas for higher doses, average changes in T_b (ΔT) remained at 45% and 65% of the maximum hyperthermic response, respectively, 180 min after the end of the perfusions. A 1-hr perfusion of PL017 in a dose of $1.69 \text{ nmol}/\mu\text{l}$ produced a $1.7 \pm 0.15^\circ\text{C}$ maximum T_b change at 60 min after the perfusions began, and the recovery time course was 180 min (fig. 6, top). A 1-hr perfusion of PL017 ($1.69 \text{ nmol}/\mu\text{l}$) followed by a 1-hr perfusion of saline or CSF induced similar T_b changes and recovery time courses (fig. 6, top).

T_b responses to reversal or blockade of PL017 with *mu* or *kappa* receptor antagonists microdialyzed into the POAH. Perfusion for 1 hr of the *mu* receptor antagonist CTAP ($0.9 \text{ nmol}/\mu\text{l}$), the *kappa* receptor antagonist nor-BNI ($1.2 \text{ nmol}/\mu\text{l}$) or the general opioid receptor antagonist naloxone ($1.3 \text{ nmol}/\mu\text{l}$) did not affect T_b (fig. 6, bottom). However, a 1-hr perfusion of CTAP or naloxone after a 1-hr perfusion of PL017 ($1.69 \text{ nmol}/\mu\text{l}$) significantly shortened the recovery time courses to 60 min or 90 min, respectively (fig. 7, top), compared with 180 min in the vehicle perfusion after the

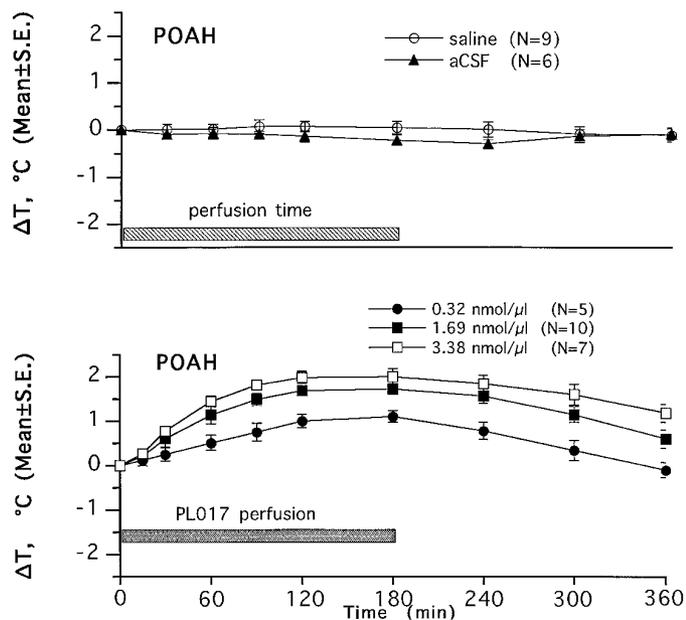


Fig. 5. Top panel) T_b changes (ΔT) of rats in response to a 3-hr intraPOAH microdialysis of pyrogen-free saline or CSF. Base-line T_b values were $37.6 \pm 0.16^\circ\text{C}$ in saline group and $37.9 \pm 0.1^\circ\text{C}$ in aCSF group. Bottom panel) Dose-related T_b changes (ΔT) induced by the selective *mu* opioid receptor agonist PL017 microdialyzed for 3 hr into the POAH of rats. Base-line T_b values were $37.8 \pm 0.2^\circ\text{C}$ in the $0.32 \text{ nmol}/\mu\text{l}$ group, $37.9 \pm 0.15^\circ\text{C}$ in the $1.69 \text{ nmol}/\mu\text{l}$ group and $37.8 \pm 0.18^\circ\text{C}$ in the $3.38 \text{ nmol}/\mu\text{l}$ group.

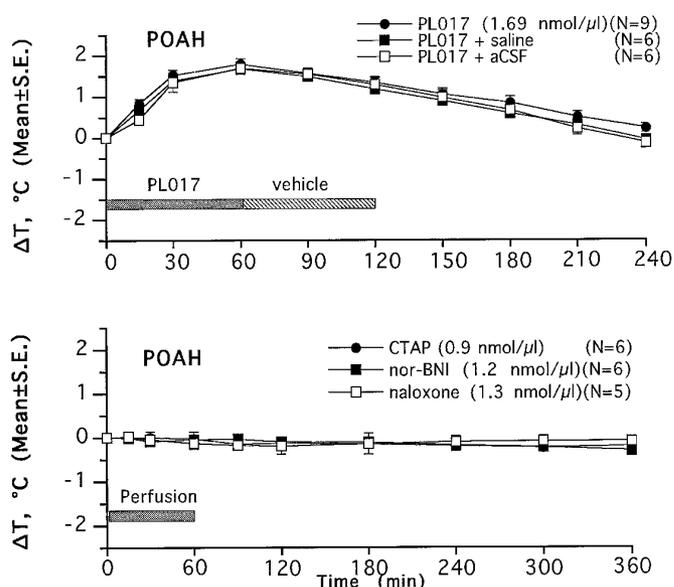


Fig. 6. Top panel) T_b changes (ΔT) of rats in response to a 1-hr intraPOAH microdialysis of PL017 ($1.69 \text{ nmol}/\mu\text{l}$) alone or a 1-hr perfusion of the same dose of PL017 followed by a 1-hr vehicle (aCSF) or saline perfusion. Base-line T_b values were $37.8 \pm 0.18^\circ\text{C}$ in the PL017 alone group, $37.6 \pm 0.16^\circ\text{C}$ in the PL017 + saline group and $37.8 \pm 0.15^\circ\text{C}$ in the PL017 + aCSF group. Bottom panel) T_b responses (ΔT) of rats to a 1-hr intraPOAH microdialysis of antagonists alone. Base-line T_b values were $37.9 \pm 0.14^\circ\text{C}$ in the CTAP group, $37.8 \pm 0.2^\circ\text{C}$ in the nor-BNI group and $37.9 \pm 0.17^\circ\text{C}$ in the naloxone group.

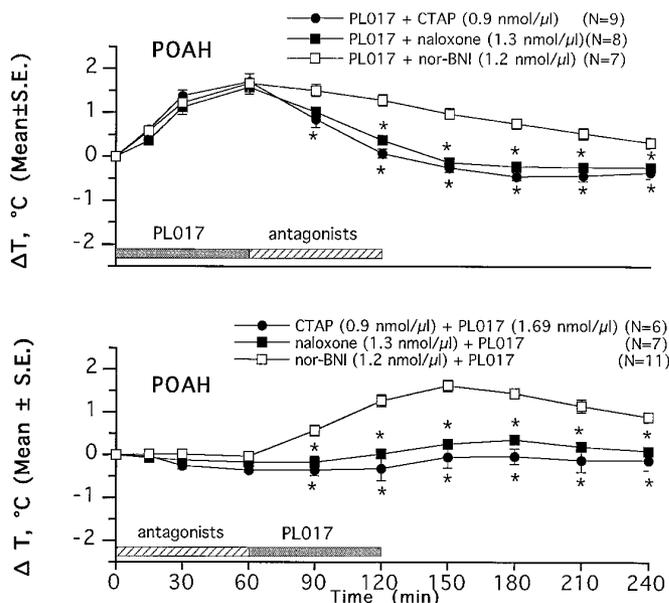


Fig. 7. Top panel) T_b changes (ΔT) of rats in response to a 1-hr intraPOAH microdialysis of PL017 ($1.69 \text{ nmol}/\mu\text{l}$) followed by a 1-hr perfusion of the *mu* receptor antagonist CTAP, the *kappa* receptor antagonist nor-BNI or the general opioid receptor antagonist naloxone. Base-line T_b values were $37.9 \pm 0.14^\circ\text{C}$ in the PL017 + CTAP group, $37.8 \pm 0.2^\circ\text{C}$ in the PL017 + nor-BNI group and $37.8 \pm 0.17^\circ\text{C}$ in the PL017 + naloxone group. * $P < .05$. Bottom panel) T_b changes (ΔT) of rats in response to a 1-hr intraPOAH microdialysis of CTAP, nor-BNI or naloxone, followed by a 1-hr perfusion of PL017. Base-line T_b values were $37.7 \pm 0.2^\circ\text{C}$ in the CTAP group, $37.8 \pm 0.17^\circ\text{C}$ in the nor-BNI group and $37.8 \pm 0.16^\circ\text{C}$ in the naloxone group. * $P < .05$.

same dose of PL017 (fig. 6, top). Nor-BNI did not change the recovery time course of PL017 (fig. 7, top). Microdialysis of CTAP or naloxone 1 hr before PL017 perfusion prevented the

hyperthermic response caused by PL017, whereas a 1-hr perfusion of nor-BNI did not block the hyperthermia but delayed the maximum response to 90 min after the onset of PL017 infusion (fig. 7, bottom).

T_b responses to the kappa receptor agonist Dyn microdialyzed into the POAH. Perfusion of Dyn for 3 hr produced a dose-related hypothermia (fig. 8). The maximum T_b changes were $-0.71 \pm 0.18^\circ\text{C}$, $-1.28 \pm 0.08^\circ\text{C}$ and $-1.48 \pm 0.09^\circ\text{C}$ for doses of 0.07 nmol/ μl , 0.35 nmol/ μl and 1.05 nmol/ μl , respectively, and they occurred 180 min after perfusions began. The recovery time courses in the responses to the three different doses were 180 min, 210 min and 240 min after the end of the perfusions, respectively. A 1-hr perfusion of Dyn (0.35 nmol/ μl) followed by a 1-hr perfusion of saline or aCSF still induced hypothermia, with a maximum T_b change of $-0.99 \pm 0.07^\circ\text{C}$ and a recovery time course of 210 min (fig. 9, top).

T_b responses to Dyn before and after CTAP or nor-BNI microdialyzed into the POAH. A 1-hr microdialysis of nor-BNI into the POAH after a 1-hr microdialysis of Dyn shortened the recovery time course to 90 min, compared with 210 min in the case of vehicle perfusion after Dyn, but a 1-hr dialysis of CTAP did not shorten the recovery time (fig. 9, top). A 1-hr perfusion of nor-BNI before Dyn prevented hypothermia; however, the hypothermia still occurred with Dyn after 1-hr perfusion of CTAP (fig. 9, bottom).

T_b responses to PL017 or Dyn microdialyzed into the PAG. Compared with the vehicle control (fig. 10, top), a 1-hr microdialysis of Dyn into PAG, in the same doses that induced hypothermia in the POAH, did not produce a significant change in T_b (fig. 10, bottom). After a 1-hr perfusion of PL017, in the same dose that caused hyperthermia in the POAH, only one dose (2.5 nmol/ μl) produced a slight increase ($0.5^\circ\text{C} \pm 0.13^\circ\text{C}$), which was not significant (fig. 10, bottom).

Analgesic responses to PL017 or Dyn microdialyzed into the PAG. A 1-hr microdialysis of PL017 in doses of 1.69 nmol/ μl and 2.5 nmol/ μl into the PAG produced 47% and 59% of maximum analgesia, respectively, on the cold-water tail-flick test, with 30% and 40% of MPA, respectively, still remaining 90 min after the end of PL017 perfusion (fig. 11, top). A 1-hr perfusion of Dyn in doses of 0.35 nmol/ μl , 0.74 nmol/ μl and 1.87 nmol/ μl into PAG induced 30%, 39% and 51% of maximum analgesia, respectively, with 15%, 24% and

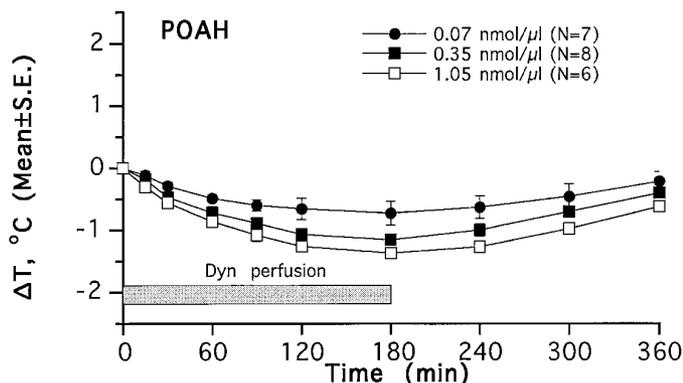


Fig. 8. Dose-related T_b changes (ΔT) of rats in response to a 3-hr intraPOAH microdialysis of Dyn in different doses. Base-line T_b values were $37.8 \pm 0.18^\circ\text{C}$ in the 0.07 nmol/ μl group, $38 \pm 0.2^\circ\text{C}$ in the 0.35 nmol/ μl group and $37.9 \pm 0.16^\circ\text{C}$ in the 1.05 nmol/ μl group.

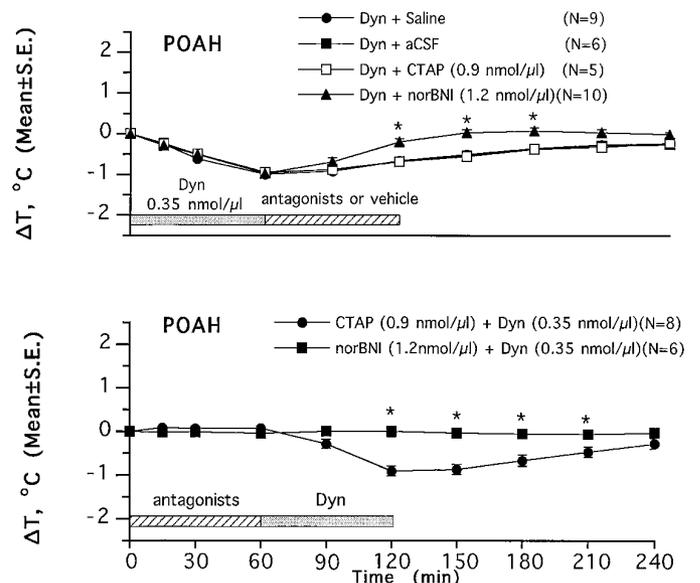


Fig. 9. Top panel) T_b changes (ΔT) of rats in response to a 1-hr intraPOAH microdialysis of Dyn (0.35 nmol/ μl) followed by a 1-hr perfusion of vehicle (saline or aCSF), CTAP or nor-BNI. Base-line T_b values were $37.9 \pm 0.17^\circ\text{C}$ in the Dyn + saline group, $37.8 \pm 0.2^\circ\text{C}$ in the Dyn + aCSF group, $37.9 \pm 0.16^\circ\text{C}$ in the Dyn + CTAP group and $38 \pm 0.17^\circ\text{C}$ in the Dyn + nor-BNI group. Bottom panel) T_b changes (ΔT) of rats in response to a 1-hr intraPOAH microdialysis of CTAP or nor-BNI followed by a 1-hr perfusion of Dyn. Base-line T_b values were $38 \pm 0.16^\circ\text{C}$ in CTAP + Dyn group and $37.9 \pm 0.2^\circ\text{C}$ in nor-BNI + Dyn group. * P < .05.

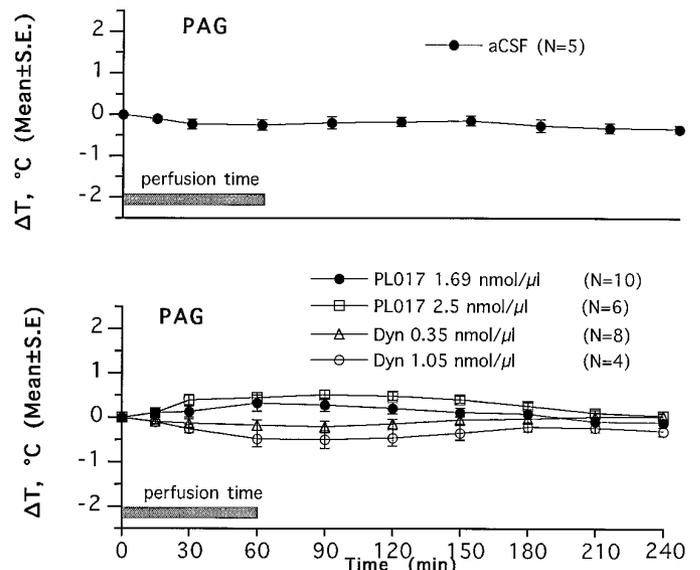


Fig. 10. Top panel) T_b responses of rats to a 1-hr intraPAG microdialysis of vehicle (aCSF). Base-line T_b value was $38.1 \pm 0.15^\circ\text{C}$. Bottom panel) T_b responses (ΔT) of rats to a 1-hr intraPAG microdialysis of PL017 or Dyn. Base-line T_b values were $37.9 \pm 0.18^\circ\text{C}$ in the PL017 1.69 nmol/ μl group, $38 \pm 0.2^\circ\text{C}$ in the PL017 2.5 nmol/ μl group, $37.8 \pm 0.2^\circ\text{C}$ in the Dyn 0.35 nmol/ μl group and $38 \pm 0.16^\circ\text{C}$ in the Dyn 1.05 nmol/ μl group.

30% of MPA observed 90 min after the end of the perfusion (fig. 11, top).

Analgesic responses to PL017 or Dyn microdialyzed into the POAH. When either PL017 or Dyn, in the same doses that induced T_b changes in POAH (1.69 nmol/ μl and 0.74 nmol/ μl , respectively), was microdialyzed into POAH,

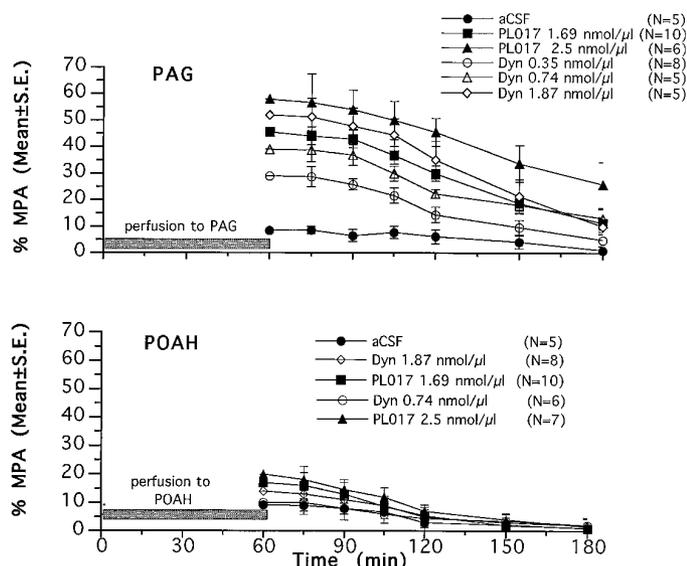


Fig. 11. Top panel) Time course of analgesic responses of rats after a 1-hr microdialysis of aCSF, PL017 or Dyn into the PAG. The points above that caused by the Dyn 0.35 nmol/ μ l group at 120 min were significant, compared with the points of aCSF group at the corresponding time ($P < .05$). %MPA: percent maximum possible analgesia. Bottom panel) Time course of analgesic responses of rats after a 1-hr microdialysis of aCSF, PL017 or Dyn into the POAH. No points of the PL017 and Dyn groups were significant, compared with the points of aCSF group at the corresponding time ($P > .05$).

neither of them produced significant analgesic responses in the cold-water tail-flick test. Higher doses of PL017 (2.5 nmol/ μ l) and Dyn (1.87 nmol/ μ l) caused maximum responses of only $20\% \pm 4\%$ and $14\% \pm 3\%$ of MPA, respectively, which were not significant ($P > .05$) compared with those in aCSF controls ($10\% \pm 3\%$ MPA) (fig. 11, bottom).

Discussion

Intracerebral microdialysis, the method used in the present study, is a way to discriminate the sites in the brain where opioids act. The drugs microdialyzed into the PAG and POAH are limited within the desired brain regions even after a 3-hr perfusion (fig. 2). Therefore, the T_b and analgesic effects can be considered the result of drugs acting directly within these regions rather than by diffusion to other brain areas. This method minimizes stress to the animals during drug administration. Although animal handling during testing may be considered a stressor, there are no significant changes in T_b or tail-flick latency from the first to the last of the measurements, as shown by the data from the aCSF control groups. Because substances pass the microdialysis probe membrane by diffusion, and there is no direct contact between the liquid flowing inside the membrane and the cells of the tissue, the acute tissue injury is less than that seen with the microinjection method, where the injector is inserted immediately before drug delivery. Also, the addition of extra volume and pressure seen with other methods of drug delivery is avoided. In the present study, after the probe was inserted into the POAH or PAG, 24 hr elapsed before the experiment was begun. This interval is important, because hyperthermia caused by an extensive mechanical lesion of the POAH fully subsides 18 hr after the lesion (Rudy *et al.*, 1977; Rudy, 1980), and local tissue perturbations that occur

immediately after implantation of a probe abate in 24 hr (Benveniste *et al.*, 1987), whereas gliosis becomes maximal in 4 days (Hamberger *et al.*, 1983). Another advantage in using intracerebral microdialysis to deliver drugs is that it is possible to maintain sterility during drug delivery because the membrane excludes large molecules, such as bacterial lipopolysaccharides, from diffusing into the brain, thereby preventing bacteria from getting to the delivery site. A disadvantage in using microdialysis to deliver high-molecular-weight peptide drugs is that they may be absorbed in the dialysis membrane or perfusion/collection tubing instead of diffusing into the target tissue, as seen with Dyn and CTAP dialyzed into the brain in the present study. However, the amount of drug delivered into the brain was still effective, because it produced T_b or analgesic responses similar to those obtained by i.c.v. drug administration in our laboratory (Adams *et al.*, 1993; Handler *et al.*, 1992; Tiseo *et al.*, 1988).

Previous results from this and other laboratories demonstrated that μ receptor agonists caused hyperthermia (Spencer *et al.*, 1988; Handler *et al.*, 1992; Adler and Geller, 1993) and κ receptor agonists produced hypothermia in rats (Adler *et al.*, 1983; Adler *et al.*, 1986; Spencer *et al.*, 1988; Handler *et al.*, 1992) after i.c.v. administration, which indicates that the thermic actions of opioids occurred in the brain. In the present study, microdialysis was used as the method of administration to facilitate drug delivery to the POAH and to restrict the administered drug to the relevant thermoregulatory site and minimize ancillary actions at other sites not directly involved in regulation of T_b . The T_b responses produced by the opioid agonists and antagonists microdialyzed into the POAH are similar to those seen in the experiments using i.c.v. administration, which demonstrates that the POAH is the crucial locus. It is not possible to determine from this study, however, whether the full effects of drugs on heat loss and heat gain are mediated solely by the receptors in the POAH.

No previous reports appear to have involved the use of highly selective opioid receptor agonists or antagonists delivered directly into the POAH. Although some showed the T_b responses by intraPOAH microinjections of morphine (Lotti *et al.*, 1966; Cox *et al.*, 1976; Trzcinka *et al.*, 1977), β -endorphin (Martin and Bacino, 1979; Tseng *et al.*, 1980; Thornhill and Saunders, 1984), the leu-enkephalin analog DADL-enkephalin (Tepperman and Hirst, 1983) and the met-enkephalin analog met-enkephalinamide (Stanton *et al.*, 1985), these drugs are not highly selective for one type of receptor. For example, although morphine is a μ -preferring opioid receptor agonist, *in vitro* binding (Magnan *et al.*, 1982) and functional (Takemori and Portoghese, 1987) assays have shown that it has low affinity for δ and κ opioid receptors, and therefore, high concentrations of morphine can activate all opioid receptors. In terms of T_b effects, a low dose of morphine acts at μ receptors and induces hyperthermia, whereas a high dose of morphine produces hypothermia (Lotti *et al.*, 1966) that is mediated by κ receptors (Adler *et al.*, 1988). β -endorphin has almost the same affinity for both μ and δ receptors (Leslie, 1987), and both enkephalins can act on μ and δ receptors (Akil *et al.*, 1984). In the present study, highly selective opioid receptor ligands, such as PL017 and CTAP for the μ receptor (Chang *et al.*, 1983; Pelton *et al.*, 1986) and Dyn and nor-BNI for the κ receptor (Chavkin and Goldstein, 1981; Takemori *et*

al., 1988), were employed to distinguish among the actions of different opioid receptors within the POAH and PAG. That highly selective opioid agonists produce opposite T_b changes that can be inhibited only by their corresponding antagonists proves the hypothesis that *mu* and *kappa* opioid receptors mediate the hyper- and hypothermic effect of opioids, respectively, and that the actions of those agonists given i.c.v. in our previous experiments occur mainly within the brain, rather than outside the brain as a result of diffusion of the drugs across the blood-brain barrier. That PL017 or Dyn microdialyzed into the POAH, but not into the PAG, induced opposite T_b changes adds to the evidence that the POAH is a primary site of opioid action on T_b and that the opioid system is involved in thermoregulation.

The neuronal characteristics of the POAH may be the basis for its important role in T_b response to opioids. The POAH contains opioid receptors (Mansour *et al.*, 1987) and the largest population of thermosensitive neurons among brain areas (Boulant *et al.*, 1989). The cold-sensitive neurons (mediating heat-gain responses) and the warm-sensitive neurons (mediating heat dissipation) within the POAH can respond to morphine by either increasing or decreasing their firing rate (Baldino *et al.*, 1980; Lin *et al.*, 1984). According to some models, the changes in firing rate alter the set point and initiate corresponding T_b responses. There is a hypothesis that two populations of thermosensitive neurons exist in the POAH, one involved in opioid-induced hyperthermia and another in hypothermia (Adler *et al.*, 1988). Previous reports from this laboratory indicated that opioid agonists could indeed alter the set point by changing the rate of metabolic heat production and by adjustments in heat exchange (Zwil *et al.*, 1988; Lynch *et al.*, 1987; Handler *et al.*, 1992). These experiments, conducted by calorimetric methods, showed that PL017 given by i.c.v. administration caused an immediate increase in metabolic heat production (oxygen consumption), resulting in increased T_b . Dyn induced hypothermia through reduction in metabolic rate.

In a result consistent with our earlier experiments using i.c.v. administration (Tiseo *et al.*, 1988; Tiseo *et al.*, 1990; Adams *et al.*, 1993), both PL017 and Dyn, microdialyzed into PAG, had analgesic effects in this study, which shows that *mu* and *kappa* receptors mediated the cold-water tail-flick response to opioids in this brain area. The PAG was the first region to be implicated in endogenous pain suppression (Reynolds, 1969) and is also believed to be a main target for morphine or other opioids to enhance descending supraspinally mediated inhibition (Basbaum and Fields, 1984). There are *mu* and *kappa* receptors within this area (Mansour *et al.*, 1987). Both enkephalin- and dynorphin-positive cells and terminals are found in the PAG (Basbaum *et al.*, 1983). The fact that microinjection of opiates into the PAG induces analgesia and inhibits the firing of the neurons within the nociceptive modulatory network, such as the dorsal horn of spinal cord and rostral ventromedial medulla (Gray and Dostrovsky, 1983; Jensen and Yaksh, 1986; Fang *et al.*, 1989; Morgan *et al.*, 1992), supports the hypothesis that opioids activate PAG output neurons by inhibiting an inhibitory interneuron (Basbaum and Fields, 1984).

The same doses of opioids administered into the PAG failed to induce significant analgesia when microdialyzed into the POAH. This result differs from a previous report (Tseng *et al.*, 1980) in which microinjection of β -endorphin into both

POAH and PAG produced analgesic and T_b responses. It is possible that the difference between these findings is due to the different methods and opioids used, because β -endorphin has almost equal affinity for *mu* and *delta* receptors (Leslie, 1987). Central administration of *delta* receptor agonists by i.c.v. (Adler and Geller 1993; Handler *et al.* 1992) or microdialysis into the PAG and POAH (Xin *et al.* 1994) does not induce any significant changes in T_b or in the metabolic parameters, which indicates that the *delta* receptor is not involved in the T_b responses to opioids under normal ambient conditions. The *delta* receptor agonist DPDPE, however, can produce analgesia in the cold-water test (Adams *et al.*, 1993).

In summary, this study has demonstrated that selective *mu* and *kappa* receptor agonists, microdialyzed into the POAH of rats, produced hyperthermia and hypothermia, respectively, and that the effects could be blocked by their corresponding antagonists given before the agonist and could be reversed if the antagonist was administered after the agonist. This is the first report of the anatomical specificity of the effects on T_b and analgesia of selective opioid receptor agonists or antagonists administered into the POAH or PAG by the intracerebral microdialysis method. It supports the hypothesis that the hyperthermic response to opioids is mediated by the *mu* receptor and the hypothermic response by the *kappa* receptor in rats. Our results also demonstrate that the POAH is a primary functional area in T_b responses to opioids and that the PAG is a sensitive area in analgesic responses to opioids. The intracerebral microdialysis method appears to be a valuable tool for investigating the effects of drugs and the interactions between drugs and endogenous chemicals in the brain.

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