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Experimental conditions for the continuous subcutaneous infusion of four central analgesics in rats

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Abstract

For the analysis of pharmacotherapeutic regimens for chronic pain in animals, it is important to establish delivery methods in which analgesics can be administered continuously and at a constant rate for a prolonged period of time. This allows for the assessment of how drug effects may vary over time in the presence of ongoing pain. The present study determined, for four analgesic compounds, the maximal doses that met all of the following criteria: (i) water-soluble, (ii) stable over 14 days at 38 °C, and (iii) devoid of undesirable side-effects in normal rats, as assessed by evolution of body weight and temperature after the subcutaneous implantation of an osmotic mini-pump that continuously infused the compounds over a 14-day period. The results showed the maximal doses to be 5 mg/rat/day for morphine hydrochloride, 2.5 mg/rat/day for imipramine hydrochloride, 20 mg/rat/day for ketamine hydrochloride, and 10 mg/rat/day for gabapentin. These doses were further found to be sufficient to express each compound's representative pharmacological activity. The conditions identified here appear appropriate for future studies of these four compounds in rat models of chronic pain and neuropathic allodynia.

Keywords: Osmotic mini-pumps; Analgesics; Subcutaneous continuous infusion; Rat; Tolerability; Pharmacological activity

1. Introduction

In addition to requiring animals models, the neurobiological analysis of chronic pain requires the definition of experimental conditions in which agents can be administered and studied not only acutely, but also with a chronicity that parallels that of chronic pain. This is both because the intrinsic actions of analgesic compounds may be highly dynamic, and because their effects may depend upon ongoing pain. Such is the case with opioids. Indeed, apparent tolerance and dependence develop, especially as opioids are administered chronically (e.g., Colpaert, 1996). Also, the magnitude of both apparent tolerance to analgesia (Colpaert, 1979; Colpaert et al., 1978, 1980) and of dependence (Colpaert, 1979; Lérida et al., 1987; Vaccarino and Couret, 1993; for review, see, Colpaert, 1996) depends on the simultaneous presence of pain. For example, studies have examined the effects of chronic, arthritic pain on the development of hyperalgesia, a sign of opioid dependence (Tilson et al., 1973); arthritic rats did not develop the decreased tail-flick latency that occurs upon chronic administration of the opioid bezitramide in nonarthritic rats (Colpaert, 1979). Also, morphine withdrawal in arthritic rats produced less squealing-upon-touch, a sign of pain, than observed in nonarthritic, morphine-withdrawn rats (Lérida et al., 1987).

Chronic drug administration in rodents can be achieved with subcutaneously implanted osmotic pumps that release drug solutions for extended periods of time (e.g., Maldonado et al., 1996). These devices deliver compounds at controlled rates that remain stable and constant over time (Bruins Slot et al., in press) and can thus be used to study a compound's dynamic actions. For example, the continuous, constant release of 0.31 mg/rat/day of the opioid fentanyl produced an initial analgesia which decayed within 8 h after pump implantation (Bruins Slot et al., in press). From 64 h onward, the continuous fentanyl release paradoxically induced hyperalgesia. These findings constitute the first evidence that proposed mechanisms of signal transduction (Colpaert, 1996; Colpaert and Frégnac, 2001) may cause persistent activation of the μ-opioid receptor to eventually

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induce a sign reversal of effects (Bruins Slot et al., in press). The osmotic pump also allows for chronic drug administration without daily, or more frequent, manipulation of the animals. To avoid such manipulation would appear particularly pertinent with animal models of chronic pain. For instance, the insertion of an intrathecal catheter was found to arrest the development of adjuvant arthritis in rats (Bervouts and Colpaert, 1984). However, a number of concerns need to be addressed before the strategy of using osmotic pumps can be implemented. The amount of compound that can be administered is limited by the compound's solubility in its vehicle. Also, a constant rate of drug delivery can be achieved only inasmuch as the drug solution remains stable at body temperature for the duration of infusion. Furthermore, the dose administered should be both pharmacologically active and sufficiently well tolerated (i.e., produce no undesirable effects).

The aim of the present experiments was to identify conditions that will render possible future studies examining the effects of chronic, continuous exposure at a constant rate to four centrally acting analgesics in rodent models of chronic pain. The following compounds belonging to distinct pharmacological classes were chosen: morphine hydrochloride as the standard μ-opioid receptor agonist (for review, see, Ossipov et al., 1999); the tricyclic antidepressant and noradrenaline and serotonin reuptake inhibitor imipramine hydrochloride (Sawynok et al., 2001; Eschalier et al., 1999); the dissociative anesthetic and noncompetitive NMDA antagonist ketamine hydrochloride (Coderre, 1999); and the anticonvulsant gabapentin (Field et al., 1997; Nicholson, 2000).

The solubility of the four compounds was determined in a single water vehicle so as to enable the parsimonious use of a single control condition. High-performance liquid chromatography (HPLC) was implemented to determine the possible degradation of the compounds after 14 days at 38 °C (i.e., as in the in vivo conditions). The next step was to verify that the dose of each compound identified as both soluble in water and stable did not produce undesirable effects in the rat; tolerability was assessed by monitoring body temperature and body weight for 2 weeks in pump-implanted rats.

Having thus defined a dose of each compound that was soluble and stable in water at 38 °C and that did not induce undesirable effects in vivo, further experiments were conducted to ensure that these doses were sufficient to express the characteristic central activity of each compound. Given that the compounds belong to distinct pharmacological classes, we chose behavioral assays that are commonly used to demonstrate the different, characteristic pharmacological activity of these drug classes, as well the experimental conditions in which they are commonly implemented. For example, although morphine exerts a variety of centrally mediated actions such as respiratory depression and reinforcing effects, perhaps its most widely documented effect is antinociception, which is often assessed by the prolongation of tail-withdrawal latency (e.g., Janssen et al., 1963). The centrally mediated actions chosen for the other compounds were the dissociative effects of ketamine (Jackson et al., 1992), the anticonvulsant properties of gabapentin (for review, see, Goa and Sorkin, 1993), and the reversal by imipramine of hypothermia resulting from tetrabenazine (TTBZ)-induced depletion of central biogenic amines (Bourin, 1990).

2. Methods

2.1. Evaluation of solubility and stability

Solubility in water was examined at concentrations of 167 mg/ml for ketamine hydrochloride, 83 mg/ml for gabapentin and imipramine hydrochloride, and 42 mg/ml for morphine hydrochloride. These concentrations were chosen (1) based on existing solubility data (see: Budavari, 1996), (2) so as to correspond with doses in mg/rat/day of base that belong to the geometrical series . . . 0.01, 0.02, . . ., 10, 20 mg/rat/day, and (3) on the basis that the volume of pump infusion was 0.12 ml/day. Thereafter, the stability of the compounds in aqueous solution was investigated at a temperature of 38 °C over 14 days; chromatographic systems described below for each compound were used to determine the purity at $T_0 + 14$ days.

2.1.1. High-performance liquid chromatography

Analyses were performed on a Merck Hitachi Lachrom HPLC apparatus consisting of an L-7100 pump, L-7450 diode array detector, L-7200 autosampler, L-7360 oven, and HSM D7000 integrator. Assays were realized using four chromatographic systems. Column (A) was a symmetry C8, 5 μm, 250 × 4.6 mm (Waters, ref. WAT054245) and Column (B) was an X-Terra RP18, 5 μm, 250 × 4.6 mm (Waters, ref. 186000496). Eluents were: (I) $CH_3CN/H_2O/KH_2PO_4$ 200/800/6.8 (ml/ml/g) adjusted at pH 4 with H_3PO_4, (II) $CH_3CN/H_2O/KH_2PO_4$ 300/700/6.8 (ml/ml/g) adjusted at pH 4 with H_3PO_4, (III) $CH_3OH/H_2O/KH_2PO_4$ 200/800/6.8 (ml/ml/g) adjusted at pH 4 with H_3PO_4, and (IV) $CH_3OH/H_2O/H_3BO_3$ 200/800/1.23 (ml/ml/g) adjusted at pH 10 with KOH. The flow rate was set to 1 ml/min and the wavelength detection to 220 nm, except for the gabapentin whose value was 202 nm. Ketamine, imipramine, gabapentin were chromatographed on Column (A) eluted with mobile phase I, II, and III, respectively. Morphine was eluted with the system IV on the Column (B) thermostated at 40 °C. All solvents were HPLC grade. Assays were realized by external standardization from a linear calibration curve.

2.2. Tolerability experiments

2.2.1. Subjects

After a 10-day quarantine period, male Sprague–Dawley rats (Iffa Credo, Lyon, France) weighing 140–160 g on

arrival were transferred to an environmentally controlled room (ambient temperature, 21 ± 1 °C; relative humidity; 55 ± 5%; 12 h light:12 h dark cycle, lights on at 7 a.m.) and housed in individual cages with standard laboratory food and water freely available. Studies were carried out in accordance with IASP guidelines and were approved by the institutional Ethical Review Committee.

2.2.2. Drug treatments

Compounds were delivered by means of an osmotic mini-pump (model 2ML2; nominal pump rate: 5 μl/h; Alza, Palo Alto, USA) that was implanted subcutaneously on the first day of experimentation, as described elsewhere (Colpaert et al., 2001). Briefly, after having shaved the skin, animals were anesthetized (Fluovac apparatus; International Market Supply, Congleton, UK) in an induction chamber with a volatile anesthetic in oxygen (isoflurane 3%). Anesthesia was maintained in a semi-open system using a nose-cone (isoflurane 2.5%). The pump was inserted through a transversal incision in the skin of the lower middle part of the back, its aperture directed towards the head. Immediately after implantation, animals were placed in a warm air (37 °C) chamber (International Market Supply) for 10 min during recovery from anesthesia. For the tolerability studies, pump removal was carried out 2 weeks later, under the same conditions as pump implantation. The site of pump emplacement was massaged daily to avoid tissue adherence.

Morphine hydrochloride (Cooperation Pharmaceutique Française, Melun, France) was administered at doses from 1.25 to 5 mg/rat/day (corresponding concentrations: 10–42 mg/ml), ketamine hydrochloride (Sigma, St. Quentin, France) was administered at 20 mg/rat/day (i.e., 167 mg/ml), imipramine hydrochloride (Sigma) was administered at 2.5 and 10 mg/rat/day (i.e., 21 and 83 mg/ml), and gabapentin (in-house extraction of active ingredient from Neurontin, Parke-Davis) was administered at 10 mg/rat/day (i.e., 83 mg/ml). Pumps that were implanted in control animals delivered 0.12 ml of 0.9% NaCl/rat per day. All compounds were dissolved in distilled water; doses refer to the free base weight. TTBZ (in-house extraction of active ingredient from Nitoman, Roche) and pentylenetetrazol (PTZ; Sigma) were administered intraperitoneally in a volume of 1 ml/100 g body weight and subcutaneously in a volume of 0.2 ml/100 g body weight, respectively.

2.2.3. Experimental design

The study was conducted in two phases, a continuous infusion phase lasting 14 days and a withdrawal phase lasting 7 days. During the 3-week experimental period, measurements were made of rectal body temperature (to the nearest 0.1 °C) by means of a thermal probe (Ellab model RM6, Carrieri Instruments, Paris, France) and of body weight (to the nearest gram).

On Day 0 of the experiment, osmotic pumps were implanted as described above. Pump treatments were as follows (doses as described above): saline, one of three doses of morphine, ketamine, gabapentin, or one of two doses of imipramine. Each experimental group consisted of $n = 7$.

Measurements were taken immediately before pump implantation on the morning of Day 0, as well as 30 min, 60 min, 2 h, 4 h, and 8 h after pump implantation. Further measurements were made once daily (at 9:00 a.m.) on Postimplantation Days 1–4 and 7–11. Two weeks later, on Day 14, measurements were made immediately before, as well as 30 min, 60 min, 2 h, 4 h, and 8 h after pump removal. Further measurements were made once daily (at 9:00 a.m.) on Postexplantation Days 1–4 and 7.

2.3. Evaluation of pharmacological activity

2.3.1. Effects of morphine in a tail-withdrawal assay

At time zero, subcutaneous osmotic mini-pump releasing either morphine (5 mg/rat/day, $n = 7$) or physiological saline (0.9% NaCl, $n = 7$) was implanted. Tail-withdrawal latency was measured 30 min, 60 min, 2 h, and 4 h after implantation. For this purpose, rats were placed in holders with their tail hanging freely outside the holder. The distal 5 cm of the tail was dipped into a container with warm water (55 ± 1 °C) and the reaction time for its withdrawal was determined to the nearest 0.1 s (Janssen et al., 1963). To avoid tissue damage, the cut-off time was 30 s.

2.3.2. Effects of imipramine on TTBZ-induced hypothermia

At time zero, subcutaneous osmotic mini-pump releasing either imipramine (2.5 mg/rat/day, $n = 7$) or physiological saline ($n = 7$) was implanted. The 2.5-mg/rat/day dose of imipramine was chosen based on results from the tolerability study (see Fig. 2B) showing the maximum soluble 10 mg/rat/day dose to severely perturb body weight gain. TTBZ (Pletscher et al., 1958) (40 mg/kg) or saline was injected intraperitoneally 15 min after pump implantation. Rectal body temperature was measured to the nearest 0.1 °C at times 60 min, 2 h, and 4 h after pump implantation.

2.3.3. Ketamine-induced state-dependence (StD)

As detailed elsewhere (Bruins Slot et al., 1999), rats were trained in daily 15 min sessions to lever press for milk reward. Access to food was limited to 20 g per day. The apparatus consisted of operant conditioning chambers that contained a lever and a liquid dipper. Reinforcement consisted of a 4-s access to the dipper that contained 0.02 ml of sweetened condensed milk. Initially, each lever press produced reinforcement (continuous reinforcement schedule), but the response requirement was gradually increased from a fixed ratio (FR) 1 to an FR10 over the course of the daily sessions.

Training began in the afternoon after subcutaneous osmotic mini-pumps releasing ketamine (20 mg/rat/day, $n = 18$) had been implanted. Training continued (cut-off: 40 sessions-to-criterion; STC) until animals reached criterion performance (i.e., completion of the first FR10 schedule of

lever-press responses within 120 s after the beginning of the session). During this training period, pumps were replaced every 2 weeks. Once animals reached criterion performance, pumps were removed on the following day and replaced with a pump containing either ketamine (20 mg/kg, $n=9$) or physiological saline ($n=9$). A period of 48 h was allowed to elapse between pump replacement and the test session. On the day of the test session, animals were tested for the recall of the response during a single 15-min test session; that is, the latency for the animal to complete a first FR10 schedule was determined. Animals were tested only once.

2.3.4. Effects of gabapentin on PTZ-induced convulsions

At time zero, mini-pumps releasing either gabapentin (10 mg/rat/day, $n=7$) or saline ($n=7$) were implanted. PTZ treatment began 4 h after pump implantation. Increasing doses of PTZ were administered subcutaneously (interval between injections: 15 min; injection volume: 0.2 ml/100 g) starting with a dose of 10 mg/kg and ending at 320 mg/kg. The increasing doses were calculated such that the total amount injected before each assessment was twofold higher than that of the previous assessment. The presence or absence of convulsions was assessed by observing the rats in their home cage between 0 and 10 min after each injection. Animals showing convulsions did not receive the further injections.

2.4. Data analysis

For body temperature (°C) and body weight (g) data, differences between the values measured before and after pump implantation (for the continuous infusion phase) and before and after pump explantation (withdrawal phase) were determined; data are expressed as area-under-the-curve (AUC) values. For the tail-withdrawal experiments, the data analyzed were log-transformed latencies. For the StD experiments, the data analyzed were log-transformed retrieval ratios, defined as the ratio of the latency to complete the first FR10 during the last training (''criterion'') session to the latency to complete the first FR10 during the test session. The TTBZ-induced hypothermia data are expressed as the difference between body temperature (°C) measured before and after the injection of TTBZ. For the anticonvulsant effects of gabapentin, the data are expressed for each animal as the log-transformed lowest dose of PTZ necessary to induce convulsions.

AUC data were analyzed using one-way ANOVA followed by the Dunnett's test, or by Student's t test when there were only two groups. For the pharmacological activity studies, tail-withdrawal and hypothermia data were analyzed using a two-way repeated-measures ANOVA followed by the Dunnett's test. The log-transformed state-dependency and PTZ-induced convulsion data, which involved only two means, were analyzed by Student's t test. ED_{50} values and 95% confidence limits were computed according to the method of Litchfield and Wilcoxon by using the PHARM/PCS program of Tallarida and Murray (1987). One-tailed tests were used for the pharmacological activity studies; the pharmacological effects of the compounds have been described previously and therefore the direction of the effect could be predicted.

Statistical significance was defined as $P<.05$.

3. Results

3.1. Evaluation of solubility and stability

3.1.1. High-performance liquid chromatography

The concentrations of 167 mg/ml for ketamine hydrochloride, 83 mg/ml for gabapentin and imipramine hydrochloride, and 42 mg/ml for morphine hydrochloride were soluble in water. These results were in accordance with solubilities of 200 mg/ml for ketamine hydrochloride, ≥ 100 mg/ml for gabapentin and imipramine hydrochloride, and 57 mg/ml for morphine hydrochloride described elsewhere (expressed as weight of the salt; Budavari, 1996) Retention times of ketamine, imipramine, gabapentin, and morphine were 6.6, 19, 10.5, and 9.5 min, respectively, in the systems described above for each compound. Linear calibration curves used to assay derivates had a coefficient of determination R^2 higher than .999. No degradation was observed for any of the compounds after 14 days in water at 38 °C.

3.2. Tolerability study

3.2.1. Saline controls

Upon the implantation of a pump releasing saline, an increase in body temperature of ≈ 0.5 °C was observed that reached peak at around 60 min (not shown). Body weight continued to increase steadily by ≈ 95 g during the 2-week chronic infusion period. Pump explantation also induced an increase in body temperature of ≈ 0.5 °C that reached peak at around 60 min. Furthermore, after an initial drop in body weight of ≈ 15 g due to explantation of the pump, animals continued to gain weight by ≈ 40 g during the week following explantation.

3.2.2. Morphine

Morphine exerted no significant effect on body temperature either during the continuous infusion phase or upon withdrawal [Fig. 1A; $F(3,24)\leq 0.77$; $P>.05$]. However, morphine-treated animals gained significantly less body weight during continuous infusion (i.e., 15% less than controls in animals treated with 5 mg/rat/day) and lost significantly more weight (i.e., 100% more than controls in animals treated with 5 mg/rat/day) upon withdrawal [$F(3,24)\geq 3.5$; $P<.05$]; post hoc comparisons showed a significant effect of the 5-mg/rat/day dose during continuous infusion, and of both the 2.5- and 5-mg/rat/day doses upon withdrawal.

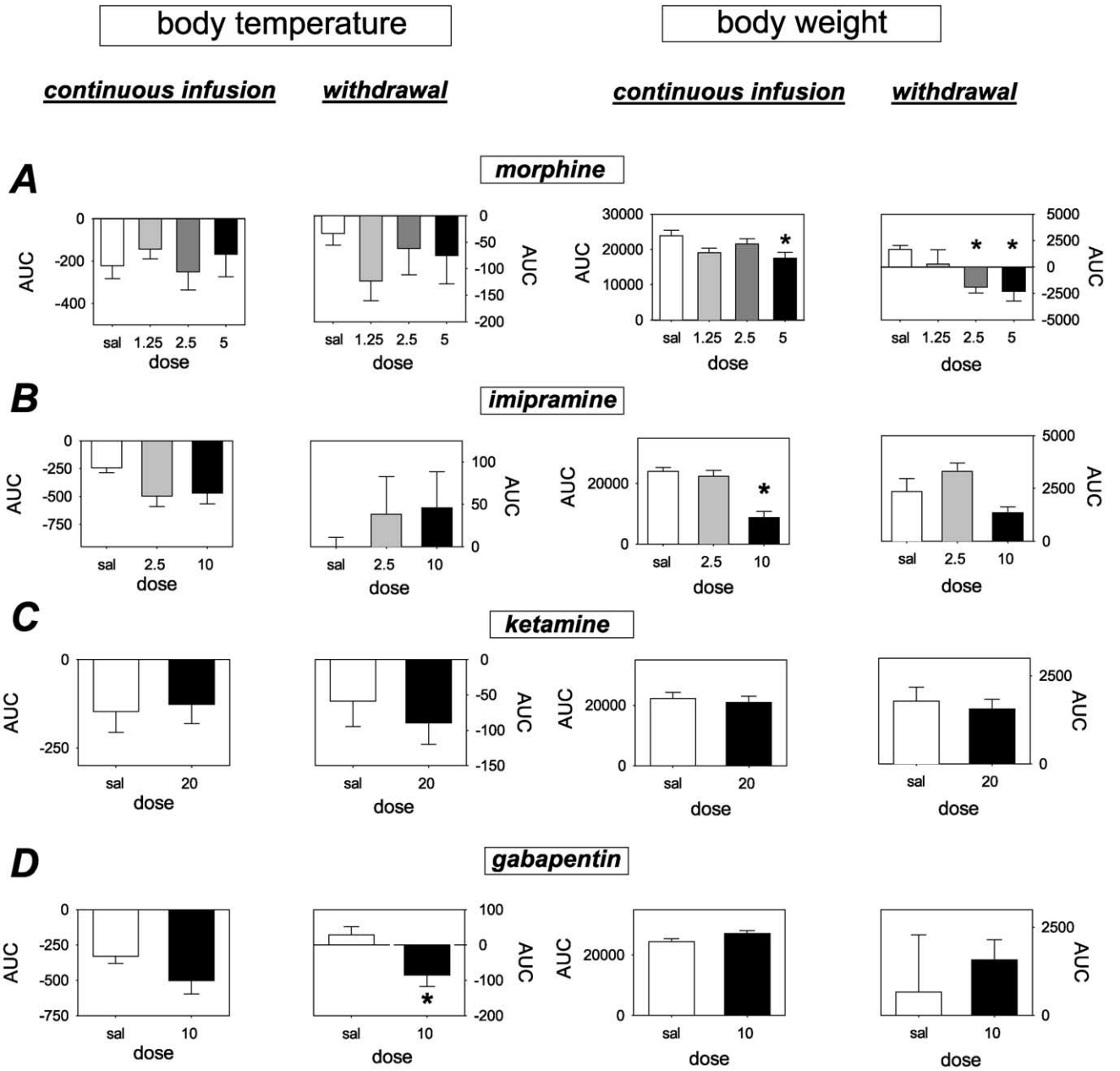

Fig. 1. Effects of continuously infused morphine, imipramine, ketamine, and gabapentin on body temperature and body weight. On the first day of the experiment, osmotic pumps were implanted subcutaneously. Pump treatments were as follows: saline; one of three doses of morphine (1.25, 2.5, or 5 mg/rat/day); one of two doses of imipramine (2.5 or 10 mg/rat/day); 20 mg/rat/day of ketamine; or 10 mg/rat/day of gabapentin. Pumps were removed 2 weeks later. For body temperature (°C) and body weight (g) data, differences between the values measured before and after pump implantation (for the continuous infusion phase) and before and after pump explantation (withdrawal phase) were determined. Data points represent AUC values (means ± S.E.M.; $n = 7$). Post hoc comparisons using Dunnett's test following one-way ANOVA or Students t test: * $P < .05$ vs. saline-treated controls.

3.2.3. Imipramine

Imipramine exerted no significant effect on body temperature either during continuous infusion or upon withdrawal [Fig. 1B; $F(2,18) \leq 3.0$; $P > .05$]. However, imipramine-treated animals gained significantly less body weight during the continuous infusion phase [$F(2,18) = 22$; $P < .001$]; post hoc comparisons showed a significant and very large effect of 10 mg/kg of imipramine. Indeed, while both saline controls and animals treated with 2.5 mg/rat/day imipramine gained about 90 g during the 2-week continuous infusion phase, animals treated with 10 mg/rat/day gained only about 45 g (i.e., 50% less). Imipramine induced an overall effect on body weight upon withdrawal [$F(2,18) = 4.8$; $P < .05$], although post hoc analysis revealed no significant difference between imipramine-treated animals and controls.

3.2.4. Ketamine

Ketamine had no effect on either body temperature or body weight either during continuous infusion or upon withdrawal (Fig. 1C; *t* test, $P > .05$).

3.2.5. Gabapentin

Gabapentin had no effect on body temperature during continuous infusion (Fig. 1D; *t* test, $P > .05$) but decreased body temperature significantly upon withdrawal (*t* test,

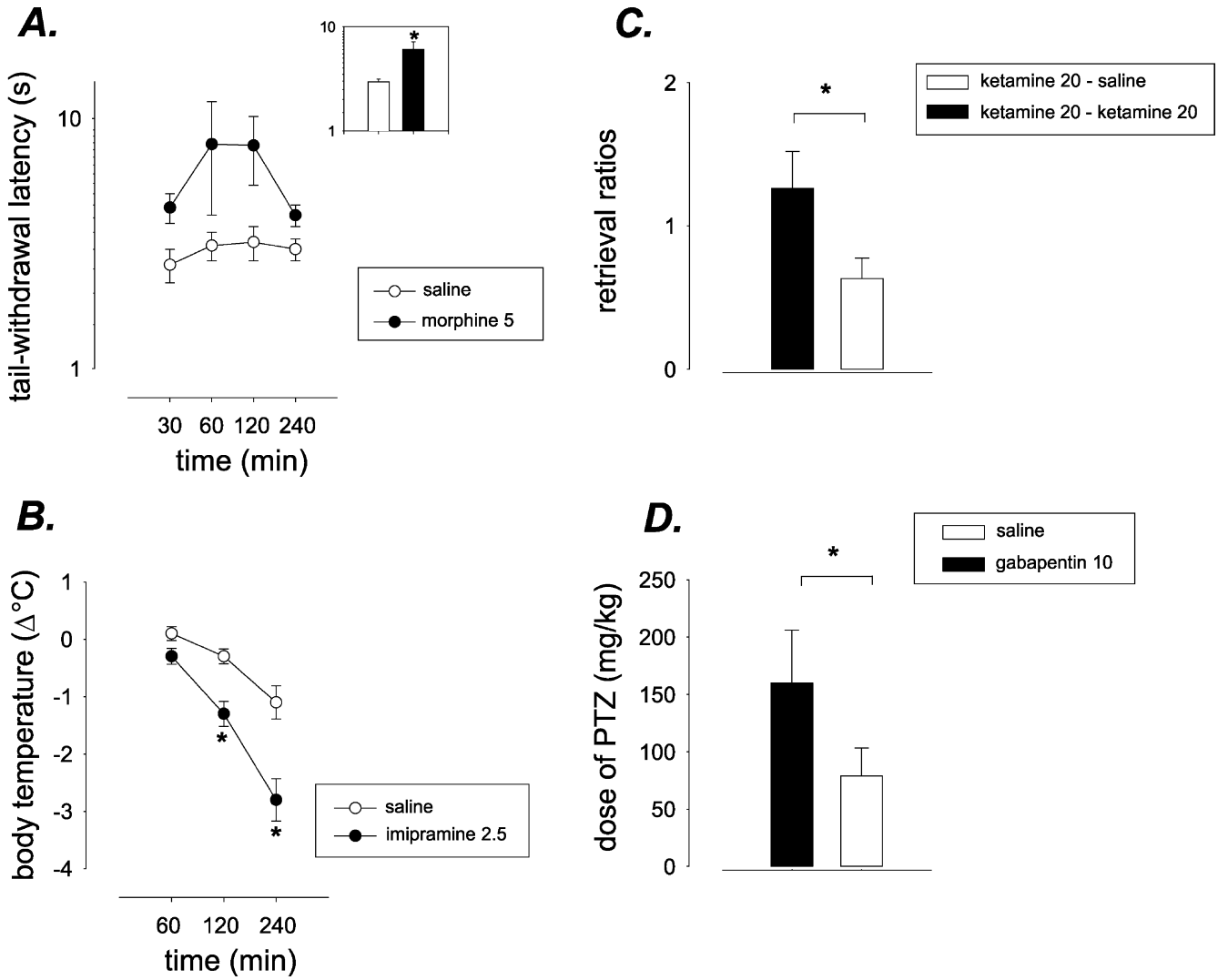

Fig. 2. Pharmacological activity of morphine, imipramine, ketamine, and gabapentin upon their subcutaneous infusion via osmotic pumps in rats. In (A), osmotic pumps were implanted subcutaneously, delivering either saline or 5 mg/rat/day of morphine. The latency (s) of the tail-withdrawal response from 55 °C warm water was determined at the stated intervals after pump implantation. Data points represent mean (± S.E.M.) values ($n = 7$). Overall means for saline- and morphine-treated animals are depicted in the inset. In (B), an osmotic pump delivering either saline or 2.5 mg/rat/day of imipramine was implanted. Fifteen minutes after implantation, all animals received an intraperitoneal injection of 40 mg/kg TTBZ. Body temperature was determined before (time 0) and at the stated intervals after TTBZ injection. Δ °C: change in body temperature that occurred between the current measure and that observed before TTBZ injection. Data points represent mean (± S.E.M.) values ($n = 7$). In (C), rats were trained in daily 15-min sessions to complete an FR10 lever-pressing task for milk-reward after having been implanted with a pump delivering 20 mg/rat/day of ketamine. Training continued until the animal completed the first FR10 schedule within 120 s after the beginning of an acquisition session (criterion latency). Once trained, pumps were removed on the following day and replaced with a pump delivering either 20 mg/rat/day of ketamine (ket 20) or saline (sal); 48 h later, a test session occurred in which the latency to complete the first FR10 schedule was again determined (test latency). Results are expressed as the log-transformed latency ratios (i.e., ratio of the criterion latency to the test latency). Data points represent geometric mean (+ upper S.E.M.) values ($n = 9$). In (D), an osmotic pump delivering either saline or 10 mg/rat/day of gabapentin was implanted subcutaneously. Starting at 4 h after implantation, increasing doses of PTZ were administered subcutaneously every 15 min, starting with a dose of 10 mg/kg and ending at 320 mg/kg. The presence or absence of convulsions was assessed by observing the rats between 0 and 10 min after each injection. Results are expressed as the average lowest dose of PTZ necessary to induce convulsions in saline- and gabapentin-treated animals. Data points represent geometric mean (+ upper S.E.M.) values ($n = 7$). Post hoc comparisons using Dunnett's test following two-way repeated-measures ANOVA: $^*P < .05$ vs. saline-treated controls. For panels (C) and (D), one-tailed Student's *t* test: $^*P < .05$.

$P < .05$). Gabapentin did not exert a significant effect on body weight during continuous infusion or upon withdrawal (t test, $P > .05$).

3.3. Evaluation of pharmacological activity

3.3.1. Effects of morphine in a tail-withdrawal assay

Morphine (5 mg/rat/day) prolonged the latency to tail withdrawal (Fig. 2A), yielding a significant main effect [inset; $F(1,12) = 6.1$, $P < .05$]. Although the analgesic effect of morphine appeared most prominent at 60 and 120 min after pump implantation, the interaction of treatment × time failed to reach significance [$F(3,36) = 1.0$].

3.3.2. Effects of imipramine on TTBZ-induced hypothermia

TTBZ induced a significant hypothermic effect that was larger in saline-treated animals compared to animals receiving 2.5 mg/rat/day of imipramine; a significant interaction of Treatment × Time was observed [$F(2,24) = 10.5$, $P < .001$]. Compared (post hoc) to saline-implanted controls, imipramine significantly reduced TTBZ-induced hypothermia at 2 and 4 h ($P < .05$) after pump implantation (Fig. 2B).

3.3.3. Ketamine-induced StD

Being infused with ketamine, the animals required an average of 11 (± 0.6) sessions to reach criterion performance (not shown). Same-state control animals that were both trained and tested with ketamine demonstrated retrieval ratios of about 1 (Fig. 2C), indicating (Bruins Slot and Colpaert, 1999) an adequate retrieval of the response acquired during training. However, changed-state animals that were trained with ketamine and tested in the presence of a saline pump showed an impairment in the retrieval of the response; analysis of log-transformed latency ratios indicated changed-state ratios to be lower than same-state retrieval ratios ($P < .05$) (Fig. 2C).

3.3.4. Effects of gabapentin on PTZ-induced convulsions

The average dose of PTZ required to induce convulsions was significantly lower in saline-treated animals as compared to animals receiving 10 mg/rat/day of gabapentin ($P < .05$) (Fig. 2D). The ED_{50} values (and 95% confidence limits) for PTZ-induced convulsions were 56.6 (27.5–116.5) and 146.8 (83.8–257) mg/kg for saline- and gabapentin-treated animals, respectively; a comparison using the PHARM/PCS program showed these ED_{50} values to be significantly different ($P < .05$).

4. Discussion

The studies reported here aimed to establish experimental conditions in which four centrally acting analgesic compounds belonging to distinct pharmacological classes can be administered chronically in the rat. For this purpose, opioid and nonopioid analgesics were continuously infused from subcutaneously implanted osmotic mini-pumps, a convenient modality of drug delivery that allows for drug delivery at a constant rate for prolonged periods of time without experimenter intervention. First, the solubility in water of morphine hydrochloride, imipramine hydrochloride, ketamine hydrochloride, and gabapentin was determined. The water vehicle was chosen to enable the parsimonious use of a single control condition throughout. Thereafter, HPLC was implemented to determine the stability of the compounds in aqueous solution after 14 days at 38 °C (i.e., as in the in vivo experimental conditions); the results showed no degradation to occur with any of the compounds.

The tolerability of the four compounds (i.e., the presence or absence of undesirable effects) was assessed by measuring body weight gain and body temperature during continuous infusion in pump-implanted animals. In saline-implanted control animals, body weight increased steadily over the 2-week continuous infusion as well as during the 1-week postexplantation period. Furthermore, an increase of about 0.5 °C in body temperature was observed in control animals following both the implantation and the explantation of the pump. Recovery from the anesthesia that was administered for both implantation and explantation included a brief 10-min period in a warming chamber at 38 °C. Experimentally naive, nonimplanted control animals demonstrated a similar increase of about 0.5–1 °C after a 10-min period in the warming chamber (not shown), suggesting the hyperthermic episode observed in saline-implanted control animals to be attributable to postanesthesia recovery. Morphine at up to 5 mg/rat/day, ketamine at 20 mg/rat/day, and gabapentin at 10 mg/rat/day appeared to be well tolerated; a marked effect was observed neither on body temperature nor on body weight during the continuous infusion phase (Fig. 1). The removal of morphine-releasing pumps 2 weeks after their implantation caused a significant and dose-dependent body weight loss (Fig. 1), confirming withdrawal-induced weight loss in morphine-dependent animals (e.g., Adams and Holtzman, 1990). Imipramine at 10 mg/rat/day very severely impaired body weight gain during continuous infusion, while the 2.5-mg/rat/day dose appeared well tolerated and was thus used in the subsequent pharmacological studies.

We next verified whether the above-defined highest soluble, stable, and tolerable concentrations were sufficient to express the distinct characteristic pharmacological activity of each compound. For morphine, a tail-withdrawal procedure (Janssen et al., 1963) was used to determine whether morphine can induce its classical opioid effect via continuous infusion by osmotic mini-pumps. Morphine at 5 mg/rat/day induced a significant effect that appeared most prominent at 1 and 2 h after pump implantation (Fig. 2A). These results are in accordance with previous data showing 10 and 20 mg/kg/day (i.e., ≈ 2.5 and 5 mg/rat/day) of continuously infused morphine to produce significant increases in tail-withdrawal latencies (Adams and Holtzman, 1990). Morphine's effect on tail withdrawal in the present experiments

(Fig. 2A) decayed as early as 4 h after pump implantation. As the development of tolerance to opioid analgesia may be counteracted by nociceptive stimulation (e.g., Colpaert et al., 1978), it will be of particular interest for future studies to examine the development of tolerance to infused morphine in rat models of chronic nociception and allodynia.

TTBZ, like reserpine, induces a depletion of central biogenic amines, i.e. noradrenaline, dopamine, and serotonin, resulting in characteristic effects such as hypothermia, sedation, and ptosis. Substances that increase the central levels of these amines (e.g., via reuptake inhibition) will prevent these effects; thus, depletion-induced hypothermia constitutes a commonly used assay to determine the actions of reuptake inhibitors (Bourin, 1990). We thus investigated whether the continuous infusion of imipramine by means of osmotic mini-pumps could antagonize TTBZ-induced hypothermia. TTBZ induced a significant decrease in body temperature in saline-implanted controls; this hypothermic effect was significantly reduced in animals receiving 2.5 mg/rat/day of imipramine (Fig. 2B).

The noncompetitive NMDA antagonist ketamine, a dissociative anesthetic, induces symptoms in man such as perceptual changes and a reduced ability to differentiate between self and nonself, much as is observed in dissociative states (Krystal et al., 1994). In the rat, dissociative effects can be measured using an StD paradigm, in which animals acquire an operant response in a given state and are then tested for the recall of the response in either the same or a different state (Colpaert, 1990). Acutely administered ketamine produces dose-dependent state-dependency in this paradigm (Jackson et al., 1992). The results obtained in the present studies demonstrate that 20 mg/rat/day of continuously infused ketamine permitted learning to occur; animals acquired the response within an average of 11 sessions, comparable to the acquisition observed in a large population of saline-treated controls (Bruins Slot and Colpaert, 1999). Animals that acquired the response in the state induced by the continuous infusion of 20 mg/rat/day of ketamine readily recalled the response when a ketamine pump was reimplanted; however, ketamine-trained animals that were reimplanted with a pump delivering saline showed a significant perturbation of recall (Fig. 2C). Note that the failure to recall the response in these changed-state animals can equally be interpreted in terms of ketamine-withdrawal producing a decrease of operant behavior. This is consistent with previous studies of chlordiazepoxide suggesting that StD can in fact operate as a mechanism of dependence (Colpaert, 1990).

Lastly, gabapentin, a structural analogue of γ-aminobutyric acid (GABA), is a relatively recent antiepileptic agent, the mechanism of action of which remains unclear (Taylor, 1997). Here, we demonstrate (Fig. 2D) that the average dose of PTZ necessary to induce convulsions was significantly higher in animals receiving 10 mg/rat/day of continuously infused gabapentin as compared to saline-treated controls. These results are compatible with previous data demonstrating the anticonvulsant activity of acutely administered gabapentin in various animal seizure models, including PTZ-induced convulsions (for review, see, Goa and Sorkin, 1993).

In conclusion, we report here experimental conditions in which four opioid and nonopioid analgesics can be administered chronically and at a constant rate in the rat. Specifically, we determined the maximal doses at which morphine hydrochloride (5 mg/rat/day), imipramine hydrochloride (2.5 mg/rat/day), ketamine hydrochloride (20 mg/rat/day), and gabapentin (10 mg/rat/day) met all of the following criteria: (i) soluble in water, (ii) stable over a 14-day period at 38 °C, and (iii) well tolerated as assessed in vivo by the evolution of body weight and body temperature after the subcutaneous implantation of a mini-pump that continuously infused the compounds over a 14-day period. The so-defined doses were further shown (iv) to be sufficient to express each compound's characteristic pharmacological activity. The definition of such experimental conditions is a prerequisite to the neurobiological analysis of chronic pain in animals, as it allows the administration of these agents with a chronicity that parallels that of chronic pain and takes into account the dynamic, neuroadaptive actions of such compounds. Using the experimental conditions identified here, studies are currently ongoing in this and a host of other laboratories to investigate the dynamic neuroadaptive actions and analgesic properties of 2-week continuous infusion of these four opioid and nonopioid analgesics in a multitude of rat models of chronic nociceptive pain and neuropathic allodynia. This concerted effort is expected to establish a unique data base and unprecedented insights in the operation of these different molecular mechanisms with pains originating from heterogeneous etiologies.

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