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European Journal of Pharmacology 411 (2001) 93–106

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Effects of intravenous and intrathecal sufentanil on a C-fibre reflex elicited by a wide range of stimulus intensities in the rat

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Received 29 June 2000; received in revised form 8 November 2000; accepted 14 November 2000

Abstract

A C-fibre reflex elicited by electrical stimulation within the territory of the sural nerve was recorded from the ipsilateral biceps femoris muscle in anaesthetised, intact rats, and in anaesthetised rats whose brains had been transected at the level of the obex. The temporal evolution of the response was studied by recording recruitment curves built with stimulus intensities from 0 to 10 times threshold. Both i.v. and i.t. sufentanil resulted in dose-dependent depressions of the reflex. Increasing the stimulus intensity from 1.5 to 10 times threshold resulted in an increase in the ED_{50} from 0.58 (0.40–0.86) to 2.40 (1.87–3.31) μg/kg for i.v. sufentanil and from 0.64 (0.46–0.79) to 1.63 (1.29–3.31) μg/kg for i.t. sufentanil. With increasing stimulus intensity, the dose–response curves showed a progressive shift to the right, but this shift was only slight with the highest intensity stimuli. The ratios for the ED_{50}s for i.v. to i.t. sufentanil were near 1. Following i.v. administration, sufentanil also facilitated the C-fibre reflex and produced tonic inter-stimulus discharges. They disappeared after the i.v. injection of naloxone. In the obex-transected rats, the depressive effect of sufentanil increased, while the facilitations and tonic inter-stimulus discharges disappeared. These findings suggest that the analgesic effects of i.v. ant i.t. sufentanil are similar, probably because sufentanil is highly soluble in lipids. Sufentanil-induced facilitations relate to supraspinal actions on motor controls and/or on the descending control of nociceptive transmission.

Keywords: Nociception; C-fibre reflex; Sufentanil; Intrinsic efficacy; Facilitation; Obex-transection

1. Introduction

The choice of an opioid by anaesthesiologists is usually based on pharmacokinetic principles. Opioids with rapid elimination half-lives may be selected for brief procedures, whereas opioids with longer elimination half-lives may be selected for longer procedures. Variations in the intensities of pain elicited by the different steps in the surgical procedure, require a continuous adaptation of the plasma concentrations of the opioid (Ausems et al., 1986).

The analgesic action of an opioid depends on both its affinity for the receptor and its occupancy of the receptor. Molecules that require a lower receptor occupancy for a pharmacological effect to be achieved, exhibit a higher intrinsic activity and therefore, a higher receptor reserve (Clarke and Bond, 1998). It has been shown experimentally that opioids with high intrinsic activity are more effective during intense nociceptive stimulation (Saeki and Yaksh, 1993; Dirig and Yaksh, 1995; Abram et al., 1997) and are less affected by the development of tolerance (Sosnowski and Yaksh, 1990; Duttaroy and Yoburn, 1995).

Various methods have been used to evaluate the intrinsic activities of opioid receptor agonists: the blockade of opioid receptors by β-funaltrexamine (Mjanger and Yaksh, 1991; Morgan and Picker, 1998), the induction of tolerance (Sosnowski and Yaksh, 1990; Duttaroy and Yoburn, 1995), and an increase in stimulus intensity (Saeki and Yaksh, 1993; Dirig and Yaksh, 1995; Abram et al., 1997; Strimbu-Gozariu et al., 1993; Guirimand et al., 1995). When the level of nociceptive input increases, the number of occupied receptors required to produce a given degree of suppression increases. This mobilisation will be low and high for drugs with high and low intrinsic activity, respectively.

Only a few methods have allowed the assessment of the effects of stimulus intensity on the analgesic effect of

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0014-2999/01/$ - see front matter
PII: S0014-2999(00)00881-5

opioids. One involves recording the responses of dorsal horn neurones evoked by activating C-fibres (Le Bars et al., 1980; Dickenson and Sullivan, 1986). However, this method is long and laborious. Another pharmacological test related to nociception consists of assessing the latency of the response to noxious thermal stimulation of the tail or hindpaw (Saeki and Yaksh, 1993; Dirig and Yaksh, 1995; Abram et al., 1997). It has been suggested recently (Yeomans et al., 1996; Yeomans and Proudfit, 1996) that nociceptive responses to rapid heating of the skin are mediated by Aδ-nociceptors, whereas the responses to slower heating of the skin are mediated by C-nociceptors. Thus, it appears that changing stimulus intensity could also change the type of responses which are recorded. Since opioids preferentially inhibit responses evoked by activation of C-fibres (Yaksh, 1997), these nociceptives responses should be reduced to a greater extent than those mediated by Aδ-fibres.

One other method consists of recording in the rat the electromyographic (EMG) reflex responses elicited by electrical stimulation of cutaneous C-fibre afferents (Falinower et al., 1994). Such a simple non-invasive method for recording the C-fibre reflex allows the examination of responses to a wide range of stimulus intensities. This model has been found to be very sensitive to the administration of opioids (Strimbu-Gozariu et al., 1993; Guirimand et al., 1995).

Sufentanil is a potent μ-receptor ligand characterised by a very high lipid solubility and a high affinity to opioid receptors (Leysen et al., 1983). Following i.v. administration, sufentanil is rapidly distributed into the brain and other tissues. The half-time for equilibration between the effect sites and the plasma is 5–6 min (Scott et al., 1991), and the elimination half-life is short (Bovill et al., 1984). Consequently, the onset of analgesic action is immediate but short-lived.

Intravenous sufentanil has become widely used to supplement general anaesthesia or as primary anaesthetic agent in very high doses during cardiac surgery. More recently, the use of i.t. sufentanil has been popularised (Hamber and Viscomi, 1999). The aim of the present study was to evaluate and compare the intrinsic activity of i.v. and i.t. sufentanil on a C-fibre reflex evoked by a wide range of stimulus intensities.

2. Materials and methods

2.1. General procedure

Experiments were carried out according to the ethical recommendations of the Committee for Research and Ethical Issues of the International Association for the Study of Pain. Male albino Sprague–Dawley rats (Charles River, France), weighing 275–350 g, were housed in groups of 3–4 per cage, allowed free access to food and water with a 12 h alternating light–dark cycle, and were acclimatised to the laboratory for at least 1 week before the experiment.

During the surgical procedure, the animals were deeply anaesthetised with 2.5% halothane in 100% oxygen. The surgical procedure consisted of performing a tracheotomy, cannulating a jugular vein and, for the i.t. studies, inserting a polyethylene catheter intrathecally to the level of the lumbar enlargement of the spinal cord. The method of i.t. injection described by Yaksh and Rudy (1976) permits the delivery of small amounts of a drug into the subarachnoid space. Basically, it consists of fixing the animals in a stereotaxic frame with the head ventro-flexed by means of a metal bar, and inserting a polyethylene (PE-10) catheter 8.5 cm down the spinal subarachnoid space through a slit in the cisternal dura and arachnoid membranes. After the animals had been prepared, they were removed from the head-holder and the experimental protocol was followed.

The procedure for transection at the level of the obex also consisted of fixing the rat in stereotaxic frame with the head ventro-flexed using a metal bar. The brainstem was exposed by a slit into the dura overlying the cisterna magna. The transection at the level of the obex was then made by electrocoagulation.

After surgery, the concentration of halothane was lowered to 1.2% in 100% oxygen. Throughout the experiments, the animals were artificially ventilated and the heart rate was monitored. Respiratory rate (50 counts/min), O_2, end-tidal CO_2 (36–40 mm Hg) and halothane level (1.2%) were monitored continuously using a capnometer (Capnomac II, Datex Instruments, Helsinki, Finland). The measurements of CO_2 and halothane were made by infrared absorption and those of O_2 with a fast paramagnetic analyser. These parameters were displayed digitally and each was controlled by an alarm. Body temperature was maintained at 37 ± 0.5°C by means of a homeothermic blanket system.

2.2. Electrophysiological recordings

The methods for electrophysiological recording have been described previously (Strimbu-Gozariu et al., 1993; Falinower et al., 1994; Guirimand et al., 1995). Recordings of reflex activity evoked by electrical stimulation of C-fibres within the receptive field of the sural nerve were made from the ipsilateral biceps femoris muscle. A pair of non-insulated, platinum–iridium needle electrodes were inserted subcutaneously into the medial part of the fourth and the lateral part of the fifth toe. Reflex responses were recorded electromyographically by another pair of non-insulated platinum–iridium needles, inserted through the skin into the biceps femoris muscle.

The electrical stimuli were single square-wave shocks of 2 ms duration that were delivered once every 6 s (0.17 Hz) from a constant current stimulator. The stimulus inten-

sities and the EMG responses were fed to an oscilloscope for continuous monitoring and to a computerized system (PLS, Notocord) for on-line digitisation. The digitised EMG responses were full-wave rectified and the C-fibre evoked responses were integrated within a time window from 150 to 600 ms after the stimulus onset. In order to quantify the continuous inter-stimulus discharges which sometimes followed the C-fibre reflex, the EMG responses were also integrated within a time window from 5.50 to 5.95 s after the stimulus. The individual reflex responses were plotted either against time, to allow the study of their temporal evolution, or against stimulus intensity, to build recruitment curves. The integrals were expressed in millivolts × milliseconds and the current intensities in milliamperes. When the recruitment curves were investigated, the stimuli were applied at increasing intensities from 0 mA to 10 times the C-fibre reflex threshold.

2.3. Experimental protocol

All the individual experiments started with a control period during which the characteristics of the reflex were determined. This took place 20 to 30 min after the end of surgery and decrease in the level of anaesthesia (or after 1 h in the rats with transections at the level of the obex). At this stage, the application of 15 mA stimuli to the sural nerve territory resulted in stable reflex responses which showed minimal spontaneous fluctuations. This preliminary finding was regarded as a prerequisite before starting the pharmacological procedures.

After the stabilisation period, a control recruitment curve was built by increasing the stimulus intensity. The magnitude of the reflex responses increased monotonically and reached a plateau at high intensities. The threshold of the C-fibre reflex was determined as being the intersection of the polymodal regression curve and the abscissa. Nine recruitment curves were built: one during the control period and one every 5 min following the injection of the drug. Between the determinations of the recruitment curves, a constant level stimulus (three times threshold) was applied.

The effect and the relative potency of i.v. sufentanil on the C-fibre reflex was determined for seven doses, namely 0.01, 0.1, 0.33, 1, 2, 5 and 10 μg/kg. All of these doses were administered in saline (NaCl, 0.9%) in such a way that a constant volume of 1 ml/kg was used. The injection was completed by flushing the catheter with 0.1 ml saline. At the end of the experiment, naloxone (0.4 mg/kg) was injected intravenously and a final recruitment curve was built. Saline (1 ml/kg) was used as a control.

The effects on the C-fibre reflex of i.t. sufentanil were determined for six doses, namely 0.1, 0.2, 0.3, 0.4, 0.5 and 1 μg. All these doses were injected in a volume of 20 μl, by means of a Hamilton syringe. After injection, 5 μl of saline was used to flush the catheter. At the end of the experiment, naloxone (0.4 mg/kg) was injected intravenously and a final recruitment curve was built. Saline (20 μl) was used as a control.

In the obex-transected animals, the effects of 1 μg/kg i.v. and 1 μg i.t. sufentanil were studied with the same protocols as described above.

Each animal received just a single dose of sufentanil (5–8 animals/group). The rats were sacrificed with an overdose of pentobarbital at the end of the experiments. The actual location of the i.t. catheter was checked by a laminectomy performed at the end of each experiment; when it was not located dorsal to the lumbar enlargement, the results were disregarded.

2.4. Processing of data

In order to analyse the recruitment curve paradigm, the individual EMG responses were each expressed as percentages of the maximal C-fibre control response and the stimulus intensities were expressed as multiples of the threshold calculated during the control period. More than 20 points were used for building each recruitment curve. However, to simplify the processing of data, only 13 points, namely those for 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 9 and 10 times threshold intensity, were considered. In some cases when one of these intensities had not actually been applied during the experiment, the response was estimated by linear regression between the two nearest points. To quantify the effects of the drug, the areas under the recruitment curves (AURC) were calculated by a trapezoidal approximation for each animal and for each time before or after injection; the percentage effect (percentage decrease in the AURC) was expressed as:

$$\%\,\mathrm{AURC} = 100 \times \left(\mathrm{AURC}_{\text{control period}} - \mathrm{AURC}_{\text{after sufentanil}}\right) / \mathrm{AURC}_{\text{control period}}$$

Data from the recruitment curve paradigm were also used to compare dose–response curves corresponding to different intensities. For intensities from 1.5 to 10 times threshold, each reflex response was expressed as a percentage of the ratio: reflex $_{\text{after sufentanil}}$/reflex $_{\text{control period}}$. These data allowed 12 dose–response curves, corresponding to the 12 intensities, to be built on semi-logarithmic plots. The slopes of the dose–response lines were tested for parallelism. The relationship between the ED_{50} and stimulus intensity was tested for linearity. Dose–response lines were analysed using a linear regression method where all the data points, rather than the mean response, were used for evaluation of the ED_{50} values. The resulting dose–response curves were tested for linearity and parallelism (Kenakin, 1993). Fieller's theorem was used to determine the 95% confidence limit. Analysis of variance were used for statistical purposes. The overall results are expressed as means ± S.E.M. and differences were considered to be significant when P was less than 0.05.

2.5. Drugs

The following drugs were used: sufentanil citrate (Janssen, France), naloxone hydrochloride (Narcan®, Du Pont Pharma, Paris, France). Doses are expressed in mg and μg. All drugs were diluted in saline and administered in a constant volume: 1 ml/kg for i.v. and 20 μl for i.t. injection.

3. Results

3.1. General characteristics of the reflex responses

As described previously (Strimbu-Gozariu et al., 1993; Guirimand et al., 1995), electrical stimulation within the territory of the sural nerve elicited a two-component flexion reflex in the ipsilateral biceps femoris muscle. The first component had a short latency (20–100 ms) and a low threshold. The second had a much longer latency (around 170 ms at three times threshold), a longer duration (around 250 ms at three times threshold) and a higher threshold (5.9 ± 0.3 mA). The first component is due to activation of myelinated cutaneous afferent fibres whereas the second component is elicited by activation of unmyelinated afferent fibres (Falinower et al., 1994); accordingly, it has been termed the ''C-fibre reflex''. In the present study, we focused on this reflex, analysing the EMG response in a post-stimulus time window from 150 to 600 ms.

Fig. 1A shows a typical individual example of recordings of the reflex elicited by several stimulus intensities. The duration and amplitude of the C-fibre reflex increased as a function of stimulus intensity from 7 to 70 mA. After digitisation, rectification and integration, individual reflex responses were plotted against stimulus intensity to build a recruitment curve (Fig. 1D, black squares). The integrated responses increased as a function of stimulus intensity from threshold and reached a plateau for levels of stimulation of more than 5 times threshold. The stimulus–response relationship could be described by a power function with a dynamic component (from 1 to 5 times threshold) followed by a static component (from 5 to 10 times threshold).

This curve allowed both the threshold and the maximal reflex response to be determined. Neither i.v. (Fig. 2A) nor i.t. (Fig. 4A) administration of saline had any effect on the amplitude or duration of the reflex. This is shown by the fact that the control and ''treatment'' recruitment curves were almost indistinguishable.

3.2. Effects of intravenous sufentanil

The i.v. administration of sufentanil depressed the reflex in a dose-dependent manner. The effects of 1 μg/kg sufentanil are shown for a typical individual example, in Fig. 1B (5 min after administration) and Fig. 1C (10 min after administration); the corresponding stimulus–response recruitment curves are represented in Fig. 1D (open squares and open triangles, respectively). Following this relatively low dose of sufentanil, the C-fibre reflexes elicited by low and high stimulus intensities were depressed and facilitated, respectively. Following the depressive effect, there were EMG discharges between successive stimuli. These tonic inter-stimulus discharges developed according to a reproducible stimulus-dependent process: at first they were within the C-fibre response but they progressively increased in duration until they outlasted the C-fibre response by several seconds; eventually they continued throughout the 6 s interval between successive stimuli. As shown in Fig. 1C, the frequency of these discharges also increased with stimulus intensity. Both the depressions and the facilitations elicited by sufentanil, disappeared within a few seconds after the i.v. injection of naloxone. In order to evaluate the tonic discharge quantitatively, we integrated the EMG signal within a time window from 5.50 to 5.95 s after the stimulus onset (corresponding to a 450 ms time period, identical to the duration of the time-window used for the C-fibre reflex). This EMG signal was taken to be representative of the tonic hyperexcitability and was subtracted from the response recorded during the C-reflex time window. Such a correction procedure was applied to differentiate as clearly as possible between changes in the baseline and changes in the C-fibre reflex itself.

The analysis of the recruitment curves provided a tool for investigating the effects of opioids on the responses to a wide range of suprathreshold stimuli. The overall effects of seven doses of i.v. sufentanil (namely, 0.01, 0.1, 0.33, 1, 2, 5 and 10 μg/kg) are shown both in terms of the recruitment curves for the C-fibre reflex (Fig. 2) and the tonic inter-stimulus discharges (Fig. 3). With doses of more than 0.1 μg/kg, sufentanil immediately elicited bradycardia and decreased end tidal CO_2 which both recovered spontaneously within less than a minute. No change in the recruitment curves or inter-stimulus discharges occurred following 0.01 μg/kg sufentanil. Following doses of 0.1 and 0.33 μg/kg, the threshold remained constant but the tonic inter-stimulus discharges appeared with the higher stimulus intensities (Fig. 3C and D). A dose of 1 μg/kg sufentanil depressed the reflex elicited by low stimulus intensities (in the 1–4 times threshold range) without changing the threshold (Fig. 2E). Despite correction of the baseline to allow for the tonic discharges, this dose also induced a significant facilitation of the reflex elicited by the higher stimulus intensities (6–10 times threshold range) until 20 min after the injection. The inter-stimulus discharges appeared rapidly after the injection and were still occurring after 35 min (Fig. 3E). With higher doses of sufentanil, namely 2, 5 and 10 μg/kg (Fig. 2F,G and H), the reflex was significantly depressed for 15, 25 and 40 min, respectively, and the threshold was increased. Inter-stimulus discharges ap-

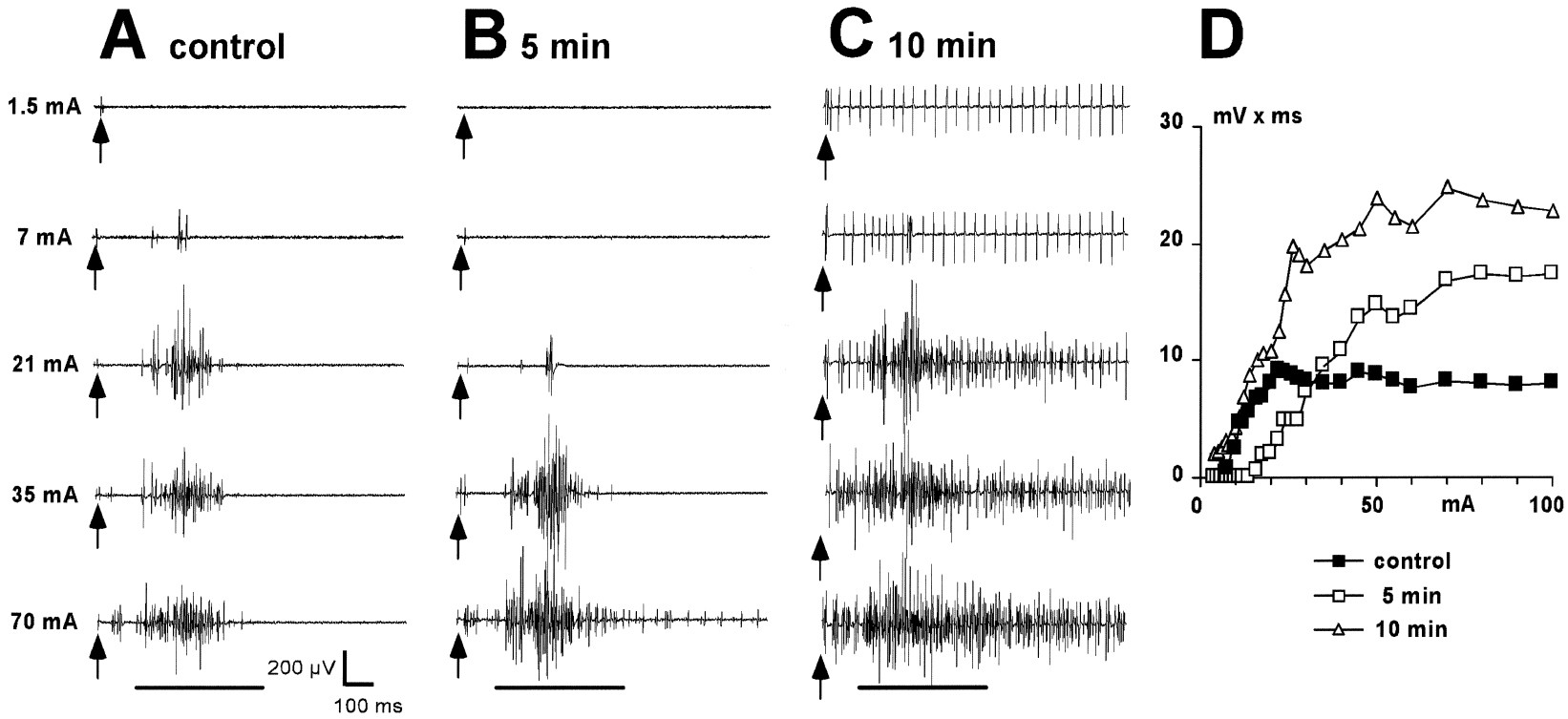

Fig. 1. Individual examples of reflex responses to C-fibre activation and the effects of 1 μg/kg intravenous sufentanil. (A) Individual EMG recordings from the biceps femoris during the control period. The responses were elicited by electrical stimulation within the sural nerve territory at the time indicated by the arrows (2 ms pulses, intensities as indicated to the left of each recording). The responses were analysed within a time-window from 150 to 600 ms following the stimuli (horizontal bar). The corresponding stimulus–response recruitment curve is shown in D (black squares). The duration and amplitude of the C-fibre reflex increased as a function of stimulus intensity and reached a maximum that plateaued at the highest levels of stimulation. (B and D (open squares)) Individual examples recorded 5 min after 1 μg/kg i.v. sufentanil. The C-fibre responses to low and high stimulus intensities were depressed and facilitated respectively. Note that with the 70 mA stimulus, the C-fibre reflex was facilitated and followed by a continuous discharge. (C and D (open triangles)) Individual examples recorded 10 min after 1 μg/kg i.v. sufentanil. Discharges were observed continuously during the 6 s inter-stimulus periods; the magnitude and frequency of these discharges increased with stimulus intensity.

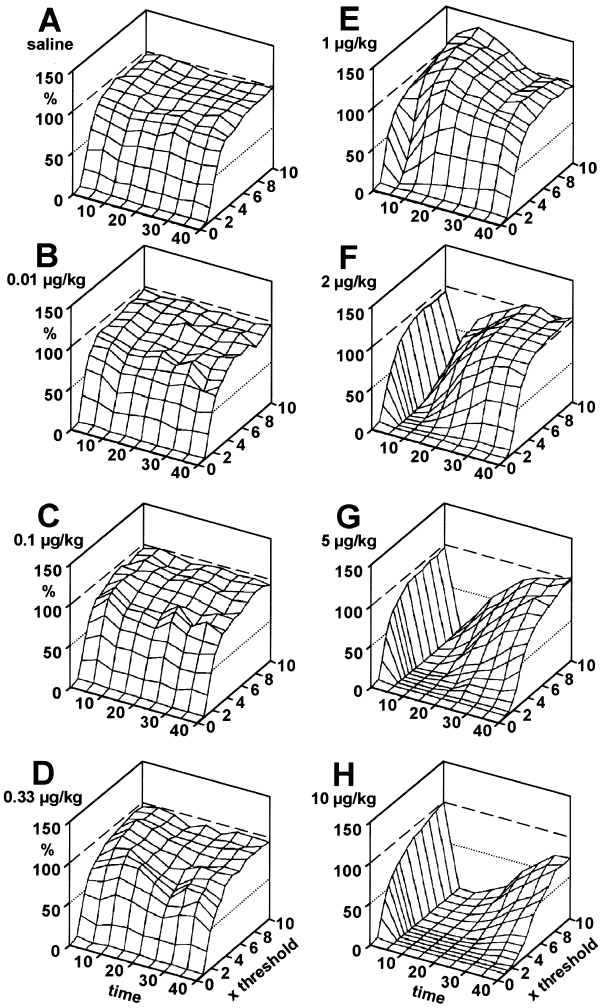

Fig. 2. Effects of saline and various i.v. doses of sufentanil on the C-fibre responses to a wide range of stimulus intensities. Nine recruitment curves were built: during the control period and every 5 min for 40 min following the injection. Abscissa: time. Ordinate: integrals of the C-fibre responses: these were calculated in a time window from 150 to 600 ms following the stimulus onset and, after subtraction of the levels of on-going activity recorded between 5.50 and 5.95 s after the stimulus, were expressed as percentages of the maximal C-fibre control responses. *Z*-axis: current intensities expressed as multiples of the threshold for the control C-fibre reflex responses. Following saline or 0.01, 0.1 or 0.33 μg/kg i.v. sufentanil (A, B, C and D, respectively), the threshold and recruitment curves remained unchanged. A dose of 1 μg/kg sufentanil (E) depressed the reflex elicited by low stimulus intensities without changing the threshold, and induced a facilitation of the reflex evoked by high stimulus intensities until the 20th min. Following 2, 5 and 10 μg/kg sufentanil (F, G and H, respectively), the reflex was depressed and showed an increased threshold until the 15th, 25th and 40th min, respectively.

peared after the maximal effect and then co-existed with the depression. Both effects lasted until the end of the experiments. Both the depressive and the facilitatory effects were completely reversed by naloxone.

3.3. Effects of intrathecal sufentanil

All doses of i.t. sufentanil above 0.2 μg, elicited a transient bradycardia. They also depressed the reflex in a dose-dependent manner for a long period of time. In addition, all doses higher than 0.3 μg induced a facilitation of the C-fibre reflex that followed the depressive effect. However, this facilitation was not associated with tonic inter-stimulus discharges: the increased EMG responses were restricted to the time-window of the C-fibre reflex. Both the depressive and the facilitatory effects disappeared a few seconds after the i.v. injection of naloxone.

Fig. 4 shows the effects of saline, naloxone (0.4 mg/kg) and six doses of i.t. sufentanil (0.1, 0.2, 0.3, 0.4, 0.5 and 1 μg). No significant change in the recruitment curves oc-

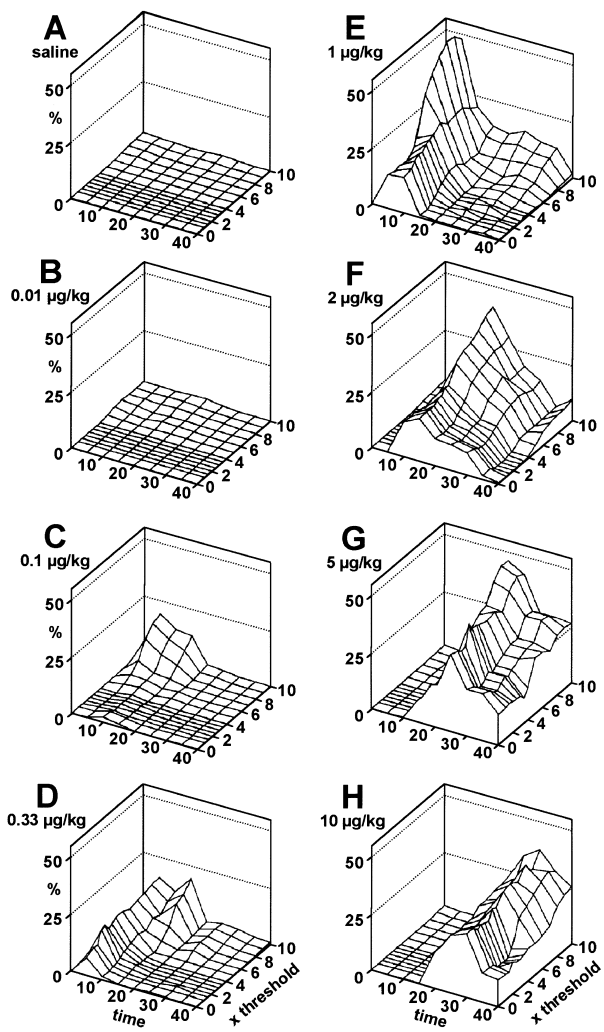

Fig. 3. Effects of saline and various i.v. doses of sufentanil on the tonic inter-stimulus discharges. In order to quantify the tonic inter-stimulus discharges following the C-fibre reflex, EMG responses were integrated within a time window from 5.50 to 5.95 s after the stimulus. Abscissa: time. Ordinate: activity expressed as a percentage of the maximal C-fibre control responses. *Z*-axis: current intensities expressed as multiples of the threshold for the control C-fibre reflexes responses. Inter-stimulus discharges were never observed after saline (A) or after 0.01 μg/kg i.v. sufentanil (B). They appeared at high stimulus intensities after 0.1 μg/kg sufentanil (C). Following 0.33 and 1 μg/kg sufentanil, the discharges developed rapidly after the injections and lasted beyond the 15th and 35th min, respectively (D and E). With high doses of sufentanil (2, 5 and 10 μg/kg), the discharges appeared following the maximal depressive effect and lasted until the end of the experiments (F, G and H).

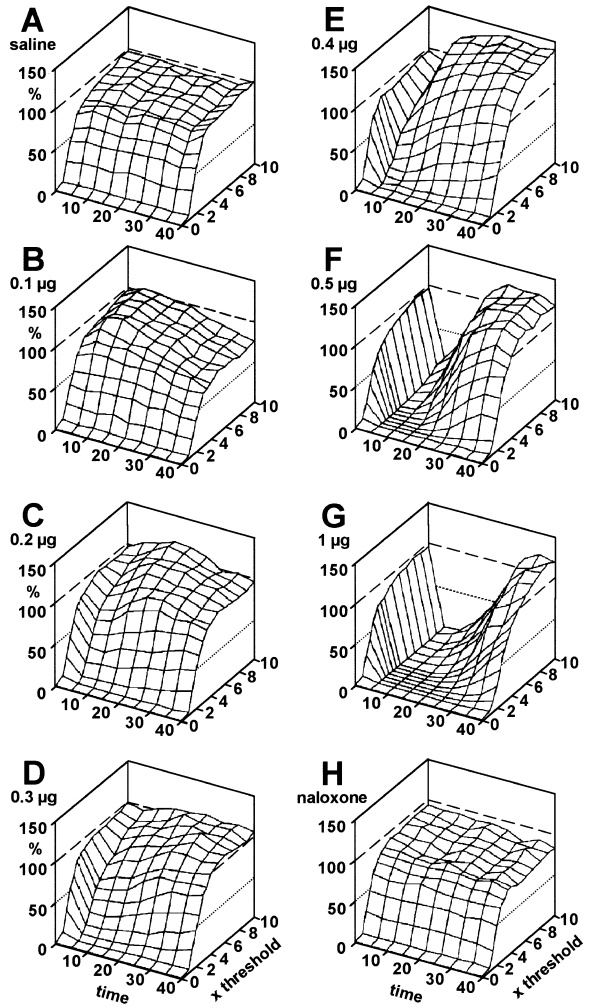

Fig. 4. Effects of saline, naloxone and various i.t. doses of sufentanil on the C-fibre responses to a wide range of stimulus intensities. Presentation as in Fig. 2. No significant change in the recruitment curves occurred after injections of saline (A), naloxone (0.4 mg/kg, H), 0.1 or 0.2 μg sufentanil (B, C). An i.t. dose of 0.3 μg sufentanil depressed the reflex elicited by suprathreshold stimuli without changing the threshold (D). Higher doses induced more complex effects, depending on both time and stimulus intensity (E, F, G). Initially, sufentanil increased the threshold and clearly depressed the reflex (until the 5th, 15th and 25th min, for 0.4, 0.5 and 1 μg sufentanil, respectively). Thereafter, the higher doses elicited a bi-directional effect which was dependent on stimulus intensity: a slight increase in threshold with a significant depression of the reflex elicited by low stimulus intensities; a significant facilitation of the response elicited by high stimulus intensities. Finally, the depressive effect disappeared although the facilitation of the responses elicited by higher stimulus intensities remained.

curred after injection of 0.1 or 0.2 μg (Fig. 4B and C). Following a dose of 0.3 μg, the reflex elicited by suprathreshold stimuli was depressed without any changes in the threshold (Fig. 4D). Higher doses induced more complex effects, depending on both time and stimulus intensity. As shown in Fig. 4E, F and G, sufentanil first increased the threshold and clearly depressed the reflex (until the 5th, 15th and 25th min following 0.4, 0.5 and 1 μg, respectively). Thereafter, higher doses elicited a bi-directional effect, which was dependent on stimulus intensity: a significant depression with a slight increase in threshold was elicited with the lower stimulus intensities whereas a clear facilitation was observed with higher intensities. Finally, the depressive effect disappeared while the facilitation of the responses elicited by the higher stimulus intensities remained. By contrast with our observations following i.v. sufentanil (Fig. 3), these facilitations were associated with few, if any, tonic inter-stimulus discharges (Fig. 5). Both the depressive and the facilitatory effects were completely reversed by naloxone.

3.4. Effect of stimulus intensity on the ED_{50} of sufentanil

The dose–response relationships for both i.v. and i.t. sufentanil, 5 min after injection, are shown in Fig. 6. For clarity of representation, only those relationships obtained with stimulus intensities of 1.5 and 10 times threshold are

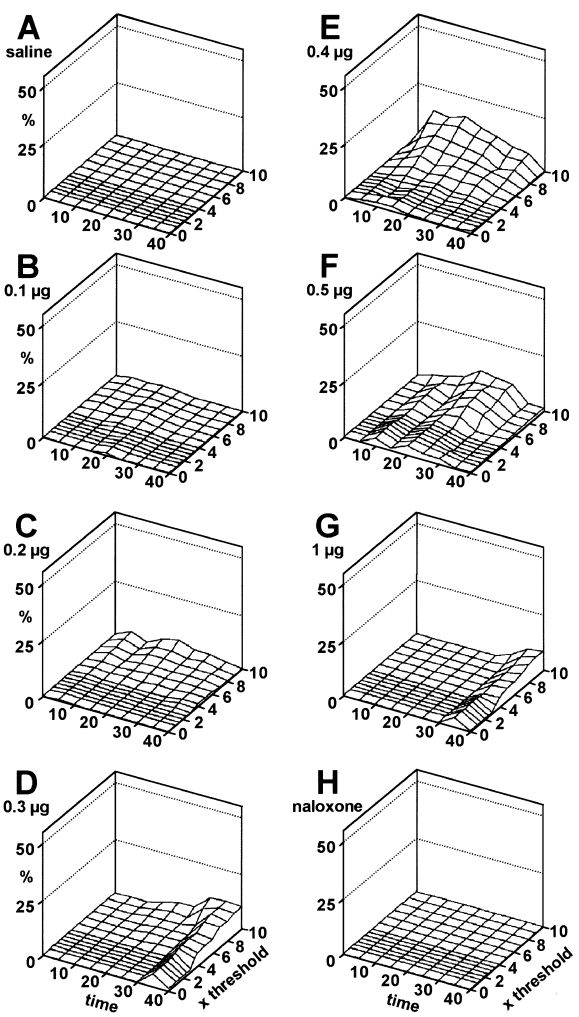

Fig. 5. Effects of saline, naloxone and various i.t. doses of sufentanil on the tonic inter-stimulus discharges. Presentation as in Fig. 3. By contrast with the effects observed following i.v. sufentanil, the facilitations resulting from i.t. administration were associated with little in the way of inter-stimulus discharges.

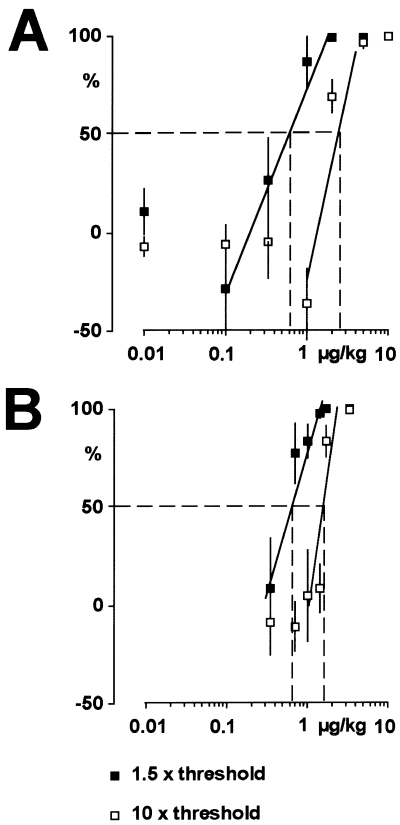

Fig. 6. Dose–response relationships obtained with the recruitment curve paradigm, using 1.5 and 10 times threshold stimuli, 5 min after the i.v. (A) or i.t. (B) injection of sufentanil. In each experiment, the areas under the recruitment curve in the 1.5 to 10 times threshold range were calculated before ($AURC_{control\ period}$) and after drug treatment ($AURC_{after\ sufentanil}$). The depression of the reflex was expressed as a percentage following the calculation: $100 \times (AURC_{control\ period} - AURC_{after\ sufentanil})/AURC_{control\ period}$. Thus a negative percentage indicates an increase in the AURC. These percentages were plotted on semi-logarithmic scales with the sufentanil doses expressed in μg/kg. Data are presented as means ± S.E.M. for each dose. The ED_{50} for these relationships are indicated by dotted lines. The relationships were steep and an increase in the stimulus intensity was associated with a shift of the relationship to the right.

illustrated. These relationships were calculated on the basis of the changes in the areas under the recruitment curves (AURC, see methods). A positive, upward, percentage indicates a decrease in the AURC, while a negative, downward, percentage indicates a facilitation with an increase in the AURC. In each case, the percentage effect exhibited a clear linear relationship with the logarithm of the dose. The effective doses that produced 50% depression were used for further analyses which were performed for all the stimulus intensities.

Although the dose–response relationships shown in Fig. 6 give a global idea of the overall effects, the methodology we developed previously (Guirimand et al., 1995) provides a means of analysing the pharmacological effects with respect to the intensities of stimulation in more detail. As explained in the methods section, 12 dose–response relationships, corresponding to 12 different stimulus intensities, were built on semi-logarithmic plots, as in Fig. 6. For i.v. and i.t. sufentanil the dose–response relationships were steep. An increase in the stimulus intensity was associated with a progressive shift of the dose–response relationship to the right.

ED_{50} were calculated for each stimulus intensity and the final results of such analyses are shown in Fig. 7. Increasing the stimulus intensity from 1.5 to 10 times threshold resulted in an increase in the ED_{50} from 0.58 (0.40–0.86) to 2.40 (1.87–3.31) μg/kg for i.v. sufentanil and from 0.64 (0.46–0.79) to 1.63 (1.29–3.31) μg/kg for i.t. sufentanil (Fig. 7A and B, respectively). A more accurate analysis of these curves revealed that the relationship between the stimulus intensity and the ED_{50} could be described with two linear relationships. If we consider the dynamic range of the stimulus–response function (1.5–5 times threshold), the ED_{50} increased rapidly. If we consider the static range of the stimulus–response function (5–10 times threshold), the ED_{50} showed a plateau.

There was very little difference in the systemic as opposed to spinal potencies: at all the stimulus intensities, the intravenous-to-intrathecal ED_{50} ratio was close to 1. Interestingly, the ratio of the ED_{50}s obtained at 10 and 1.5 times threshold were quite similar for i.v. (4.1) and i.t. (2.6) administration.

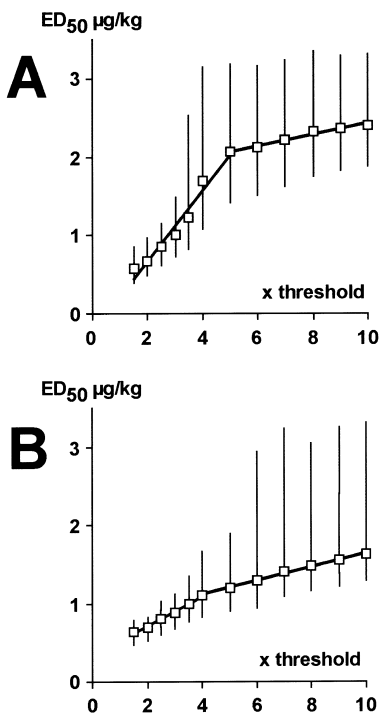

Fig. 7. Influence of stimulus intensity on the ED_{50} for i.v. (A) and i.t. (B) sufentanil, 5 min after injection. Abscissa: stimulus intensity expressed in multiples of the threshold for the control C-fibre reflex responses. Ordinate: the ED_{50} with their 95% confidence limits. Increasing the stimulus intensity from 1.5 to 10 times threshold resulted in an increase in the ED_{50} from 0.58 (0.40–0.86) to 2.40 (1.87–3.31) μg/kg for i.v. sufentanil and from 0.64 (0.46–0.79) to 1.63 (1.29–3.31) μg/kg for i.t. sufentanil. The ED_{50} increased more rapidly between 1.5 and 5 times threshold than between 5 and 10 times threshold.

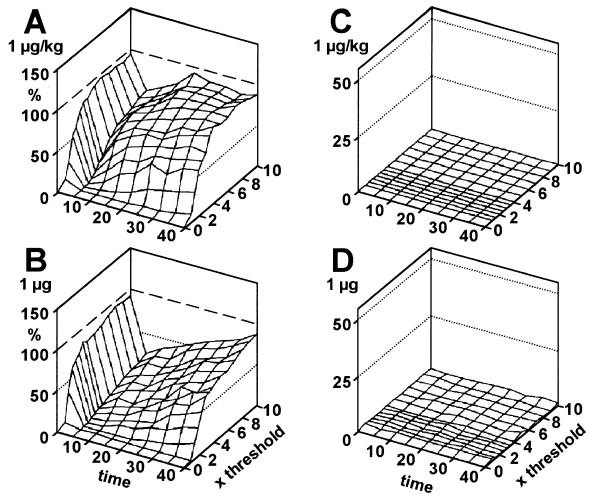

Fig. 8. Effects of 1 µg/kg i.v. (A) and 1 µg i.t. (B) sufentanil in obex-transected animals on the C-fibre responses to a wide range of stimulus intensities and on the tonic inter-stimulus discharges. Presentation as in Figs. 2 and 3. By contrast with the data from intact animals (Figs. 2E and 4G), only a depressive effect was observed during the first 10 or 30 min following the injection of 1 µg/kg i.v. or 1 µg i.t. sufentanil, respectively (A, B). Facilitations and inter-stimulus discharges (C, D) were not recorded at any time or at any stimulus intensity.

3.5. Effects of sufentanil in obex-transected animals

The C-fibre reflexes of obex-transected animals were generally weaker and less well time-locked than those seen in the intact animals. Their thresholds were lower (4.3 ± 0.2 mA) than in the intact group (5.9 ± 0.3 mA), but their latencies and durations were similar.

The effects of 1 µg/kg i.v. and 1 µg i.t. sufentanil on the recruitment curves are shown in Fig. 8A and B, respectively. Note that only a depressive effect was observed: facilitations and inter-stimulus discharges (Fig. 8C and D) were never recorded at any time or with any stimulus intensity. Sufentanil clearly depressed the reflex for a longer time period than in intact animals (Figs. 2E and 4G).

4. Discussion

In this study, i.v. or i.t. administration of sufentanil resulted in a dose-dependent depression of a nociceptive flexion reflex elicited by activation of C-fibre afferents in the sural nerve of anaesthetised rats. However, following i.v. administration, sufentanil also elicited a facilitation of the C-fibre reflex that occurred together with tonic inter-stimulus discharges. Following i.t. administration, facilitation occurred only after higher doses and was not accompanied by any obvious tonic inter-stimulus discharges. In obex-transected rats, the depressive effect of sufentanil increased while facilitations and tonic inter-stimulus discharges disappeared. We will discuss successively the depressive effects, the intrinsic activity of the two routes of administration and the facilitatory effects of sufentanil.

4.1. Depressive effects of sufentanil

Sufentanil is highly selective for µ-opioid receptors (Leysen et al., 1983; Colpaert et al., 1986), which probably reduce dorsal horn nociceptive neuronal activities via both pre- and post-synaptic mechanisms (Yaksh, 1997). Although our knowledge of the circuitry of the polysynaptic network between primary afferent neurones and flexor motoneurones is far from complete, we can assume that it involves at least dorsal horn convergent neurones (Schouenborg et al., 1995; Jasmin et al., 1997). The increase in responses with stimulus intensity probably reflects both an increase in the activity of interneurones and the recruitment of parallel polysynaptic connections with latent synapses (Carstens and Ansley, 1993; Carstens and Wilson, 1993). Interestingly, the depressive effects following i.v. or i.t. administration of various opioids are a common characteristic of both convergent neurones and C-fibre reflexes (Strimbu-Gozariu et al., 1993; Guirimand et al.,1995; Yaksh, 1997).

Our results show that sufentanil either depressed or totally suppressed the C-fibre reflex. Increasing the dose resulted in two successive effects: first, a reduction in the slope of the recruitment curve, which corresponded to a decrease in the gain of the stimulus–response function and then, an increase in the threshold. Two such phases are reminiscent of those reported following i.t. or i.v. morphine (Strimbu-Gozariu et al., 1993; Guirimand et al., 1995). The C-fibre reflex was completely blocked within 5 min following 1 µg i.t. or 5 and 10 µg/kg i.v. sufentanil. Such a complete blockade of all nociceptive signals at the spinal level, confirms the ability of sufentanil at appropriate doses, to produce complete analgesia during the course of anaesthesia.

These data are in agreement with human studies showing that morphine produces a powerful depression of the nociceptive R_{III} flexion reflex that parallels the relief of pain (Willer and Bussel, 1980): after meniscus surgery, epidural morphine increased the threshold and decreased the slope of the stimulus–response function. In paraplegic patients with total spinal cord sections, i.v. morphine also depressed the nociceptive R_{III} reflex (Willer et al., 1985), suggesting that this is a direct spinal effect.

We studied the effects of sufentanil on the C-fibre responses to a wide range of stimulus intensities in obex-transected rats. The obex is caudal to the vestibular nuclei and a section at this level avoids both spinal shock and the rigidity of decerebration (Lundberg, 1982). Both i.v. and i.t. administration of sufentanil depressed the nociceptive flexion reflex in the obex-transected animals but for a longer period of time than in the intact animals. Such an increased potency of sufentanil suggests that its direct spinal action was influenced by the obex transection. It is conceivable that the direct spinal depressive effect of sufentanil was partially inhibited in the intact rats by descending supraspinal controls and that the obex transec-

tion removed this inhibition and potentiated the antinociceptive effect.

It is often accepted that opioids potentiate the descending inhibitory mechanisms that control the spinal transmission of nociceptive inputs to the dorsal horn (Fields and Basbaum, 1994). However, several studies did not support this assertion. For example, i.t. morphine was found to be more effective in spinal than in intact rats when assessed with the tail-flick test (Siuciak and Advokat, 1989). An electrophysiological study reported that the dose–response curves for systemic morphine were similar in these two preparations (Le Bars et al., 1980). The contradictory results on the role of descending supraspinal controls in opioid analgesia are probably related to the type of preparation used. Indeed, spinal transection dramatically changes the receptive fields for nociceptive withdrawal reflexes (Schouenborg et al., 1992) and the distribution of morphine in the central nervous system following systemic administration (Advokat and Gulati, 1991).

4.2. Intrinsic activity of sufentanil

Compared to most pharmacological tests related to nociception and analgesia, which involve either threshold measurements or responses to a single suprathreshold stimulus, the present study involved another key parameter, namely stimulus intensity. Information regarding the effects of drugs on the responses to threshold and suprathreshold stimuli can easily be obtained by building stimulus–response recruitment curves. In addition, recording the C-fibre reflex is a simple non-invasive method which requires minimal surgical preparation, and thus avoids some of the bias that may be introduced for example by the laminectomies, which are unavoidable when recording from spinal neurones (Hartell and Headley, 1996).

By investigating the effects of i.v. and i.t. sufentanil on responses elicited by a wide range of suprathreshold stimuli, we found that the dose–response curves were shifted to the right when the stimulus intensity increased. These results could be interpreted in the light of the theory of drug's intrinsic efficacy including an interaction between the intrinsic activity and the stimulus intensity. According to this theory, the magnitude of the pharmacological effect of a drug is proportional to both the receptor occupancy and the magnitude of the response: when the stimulus increases, the response increases and more receptors have to be occupied in order to achieve an identical pharmacological effect. An agonist that produced antinociceptive effects with low receptor occupancy exhibits a high intrinsic activity (Kenakin, 1993; Clarke and Bond, 1998). When nociceptive inputs increase, the number of occupied receptors must be higher for a given reduction of the response to be achieved, thus the dose–response curve shifts to the right. Importantly in this respect, sufentanil completely blocked the C-fibre reflex even when it was elicited by very high intensities of stimuli (10 times threshold).

Interestingly, the ED_{50} for i.v. morphine was determined previously with the same method and was reported to increase linearly from 2.6 to 6.3 mg/kg within a range of stimulus intensities from 1 to 7 times threshold; higher intensities of stimuli were not tested in that study (Guirimand et al., 1995). To some extent, the effect of sufentanil was different in that it was possible to describe two phases: with low stimulus intensities (1–5 times threshold), the ED_{50} increased in an intensity-dependent manner, whereas at higher stimulus intensities (5–10 times threshold), it showed a plateau. These two phases corresponded to the dynamic and static components of the control stimulus–response function, respectively. Such observations suggest close dynamic relationships between physiological (the control stimulus–response curve) and pharmacological (the stimulus–effect function) properties of the network with which we are dealing. In other words, we have described a close inverse relationship between the control response and the pharmacological effect. When the stimulus intensity reached the threshold for a maximal response, there was no need to further increase the dose of sufentanil for a full effect to be achieved.

Such observations suggest that the response to a low level of nociceptive information arriving in the spinal cord can be blocked by a partial occupancy of opioid receptors. At high stimulus intensities, the small nature of the right-shift of the dose–response curve probably reflects the high intrinsic efficacy of sufentanil, whether administered via the systemic or the intrathecal route.

By comparison with morphine (Guirimand et al., 1995), sufentanil was found to be more efficacious in blocking the nociceptive responses elicited by a strong stimulus, probably because of its high intrinsic activity. Interestingly in this respect, sufentanil has successfully replaced morphine for severe postoperative pains or intractable chronic cancer pains even in morphine-tolerant patient (De Leon-Casasola and Lema, 1992).

During anaesthesia, opioids are administered by intermittent bolus injections or by continuous infusion. This latter mode of administration provides a more stable plasma concentration and a more stable anaesthetic regimen. However, the intensity of nociceptive stimulation varies during the operative procedure. For alfentanil, it has been shown that different perioperative stimuli require different concentrations to suppress undesirable responses (Ausems et al., 1986). For sufentanil, the plasma concentrations required for adequate anaesthesia have not yet been determined. Since variations in the sufentanil ED_{50} at higher stimulus intensities were low in the present study, it is possible that changes in drug infusion rate will not be necessary during increases in the intensity of the nociceptive input triggered by the surgical procedure, provided the rate is sufficient to cover the whole range of stimulus intensities. Clinical investigations with the same anaes-

thetic procedure will be necessary to check this hypothesis and determine the minimal rate sufficient for any procedures.

Various methods have been described for quantifying intrinsic efficacy by modifying the fractional occupancy of receptor sites, i.e. by reducing the receptor reserve. In an initial approach, an irreversible blockade of a part of opioid receptors is achieved by the administration of irreversible antagonists such as β-funaltrexamine (Mjanger and Yaksh, 1991; Morgan and Picker, 1998). In a second approach, induction of tolerance by repeated administration of opioids elicits a reduction of the receptor reserve and therefore allows a comparison between opioids. Agents with high efficacy such as sufentanil will leave more receptors in the non-tolerant state and produce a smaller degree of tolerance than will agents with low efficacy (Sosnowski and Yaksh, 1990; Duttaroy and Yoburn, 1995). Changing the intensity of the nociceptive stimulus provides another method for mobilising the receptor reserve. When the level of nociceptive input increases, the number of occupied receptors required to produce a given effect also increases. This mobilisation will be lower for drugs with higher intrinsic activities (Saeki and Yaksh, 1993; Dirig and Yaksh, 1995; Abram et al., 1997; Strimbu-Gozariu et al., 1993; Guirimand et al., 1995).

Unfortunately, most of these studies used behavioural tests in which the only parameter monitored was the latency of a nociceptive response to a low or high intensity noxious stimulus (tail flick, limb withdrawal...). In such cases, a threshold measurement is the set-point, with the stimulus being applied for a longer period of time when a lower intensity is employed. We do not know whether or not there are any functional differences between the response to low intensity (but long duration) and high intensity (but brief) stimuli. For example, it has been suggested that high-intensity radiant heat activates peripheral Aδ-fibres while low-intensity radiant heat activates peripheral C-fibres (Yeomans et al., 1996; Yeomans and Proudfit, 1996). In this case, changing the stimulus intensity also changes the type of response which is recorded. Since opioids strongly depress responses due to C-fibres and, to a lesser extent, those due to Aδ-fibres (Yaksh, 1997), such comparisons are essentially meaningless.

Our results differ little from studies previously published on sufentanil (Dirig and Yaksh, 1995; Abram et al., 1997). However, for calculation of the effect of a drug, the authors used the so-called ''maximum possible effect'': % MPE = (post-drug latency − control latency) × 100/(cut-off time − control latency). The calculation of this index introduces an arbitrary ratio which is dependent on the choice of cut-off time by the investigator. Thus, simply reducing the cut-off time can improve the efficiency of a substance! In the thermal nociceptive test, the time limit varies from 6 to 20 s. Thus, an increase of latency from 3 to 6 s will give a % MPE of 18%, 43% or 100% depending on whether a cut-off time of 20, 10 or 6 s was chosen. ''Maximum decided effect'' would seem to be a much better statement than ''maximum possible effect''.

4.3. Comparison of i.v. and i.t. sufentanil administration

Within the context of our experimental model (anaesthetised animals, electrical stimulation, hindlimb reflex), our results show that the ED_{50} ratios for i.v. to i.t. sufentanil were near 1. For morphine it was 10,000 (Strimbu-Gozariu et al., 1993; Guirimand et al., 1995). Intravenous, epidural and intrathecal sufentanil were found to be equipotent in producing antinociceptive and supraspinal effects in the rat (Colpaert et al., 1986; Boersma et al., 1992). Sufentanil is a drug which is highly soluble in lipid, with a rapid onset and short duration of action. Following i.v. administration, sufentanil diffuses rapidly within the central nervous system (Colpaert et al., 1986), while after i.t. administration, it is quickly absorbed into the plasma (Hansdottir et al., 1991). The bradycardia observed following i.t. sufentanil in both our study and in human subjects (Houweling et al., 1992), probably resulted from a rapid systemic redistribution and the supraspinal effects of the drug. Thus, our data do not support there being an ''obvious'' advantage in replacing systemic with i.t. sufentanil administration (as is the case with morphine). Usually, opioids are associated with a local anaesthetic. In one study (Camann et al., 1992), authors described a better effect of i.t. 10 μg sufentanil alone during labour. However, this study was based on a small number of patients, some multiparous and some nulliparous and some in spontaneous labour while others had been induced.

4.4. Facilitatory effects of sufentanil

Following the expected depression, sufentanil elicited naloxone-reversible facilitations of the C-fibre reflex. A similar observation had previously been made for morphine in rats (Guirimand et al., 1995) and monkeys (Yeomans et al., 1995). Following i.v. sufentanil, facilitations of the C-fibre reflex occurred together with tonic interstimulus discharges. The sufentanil-induced facilitation of the C-fibre reflex was intensity-dependent with no effects at threshold level. No EMG activities were recorded in the absence of electrical stimulation. Facilitation and interstimulus discharges were never observed in obex-transected rats, suggesting a supraspinal effect of the drug. The fact that i.t. sufentanil, unlike morphine (Yaksh and Rudy, 1977), spreads rapidly toward the brain (Boersma et al., 1992) supports this conclusion. Indeed, the sources of such effects could include supraspinal sites of action and involve a modulation of the descending pathways that control both the motor and sensory facets of the reflex arc.

Opioids in high doses induce intense generalised muscle rigidity, especially during induction of anaesthesia (De Lange et al., 1982) and more rarely in the postoperative period (Bowdle and Rooke, 1994). Kuschinsky et al. (1977)

recorded spontaneous extensor α-motoneurone reflex discharges in the rat, and observed a naloxone-reversible facilitatory effect with high doses of systemic morphine, which was blocked by spinalisation. Multiple brain sites could play a role in opioid rigidity. It has been suggested that the activation of opioid receptors in the substantia nigra and striatum produce muscle rigidity via a decrease in striatonigral GABAergic (gamma-aminobutyric acid) transmission (Turski et al., 1984). Other areas of the brain through which opioids have been reported to cause rigidity are the periaqueductal gray and the nucleus raphe pontis (Weinger et al., 1991). It has been shown that serotoninergic receptor antagonists (Weinger et al., 1987) and α_2-adrenoceptor agonists (Weinger et al., 1995) antagonise opiate-induced muscle rigidity. Opioids appear to produce muscle rigidity via activation of central μ_1-opioid receptor (Reisine, 1995). Behavioural studies have shown that high doses of sufentanil (even given intrathecally) can induce motor dysfunction, muscular rigidity, catalepsy and sometimes cyanosis (Yaksh et al., 1986).

These effects suggest that some results obtained from unanaesthetised animals in which the latency of a movement is used as a set point, must be interpreted with caution because increases in latency of a movement resulting from rigidity cannot be distinguished from antinociceptive effects (Miaskowski et al., 1991). These effects could also explain some discrepancies between pharmacological studies reporting increases in EMG activities and blockades of movement, which could be misinterpreted as pro- and anti-nociceptive effects, respectively (Yang et al., 1992).

In our study, facilitation of the reflex was induced by doses of sufentanil, which were clearly too low to produce rigidity, catatonia, catalepsy or stiffness of the tail. However, they were sufficient to modify the muscular tone by increasing the amplitude of contractions of the muscle in response to stimulation. It is possible that under our experimental conditions, the facilitation of the C-fibre reflex, and the tonic inter-stimulus discharges, were related to such an action of sufentanil on the regulation of motor systems.

An alternative or complementary hypothesis involves the sensory part of the reflex arc. Electrophysiological studies indicate that opioids can elicit both inhibitory and excitatory effects (Smart and Lambert, 1996). Stimulation of opioid receptors can activate both inhibitory and facilitatory nociception systems, but the excitatory effects of opioids occur in both nociceptive (Dickenson and Sullivan, 1986; Mokha, 1993) and non-nociceptive neurones (Jones et al., 1990). Some of these neurones could be interneurones for the C-fibre reflex arc. Excitatory effects of opioids probably involve several mechanisms. Electrophysiological studies of mouse dorsal root ganglia neurones in vitro revealed that micromolar concentrations of opioids evoked a reduction while nanomolar concentrations elicited a naloxone-reversible prolongation of the duration of the action potential (Shen and Crain, 1989). It has been shown for neurones in the spinal trigeminal nuclei that the opioid receptor agonist DAGO (D-Ala$_2$-MePhe$_4$-Glyol$_5$) increased the efficacy of the NMDA receptor-mediated glutamate response via a protein kinase C mediated removal of the Mg^{2+} blockade of the NMDA receptor channel (Chen and Huang, 1991, 1992). In this case, even small amounts of excitatory amino acid may have activated the NMDA receptor, which is well known to have a key role in hyperalgesia (Mao et al., 1995). In vivo, it has recently been demonstrated that pain facilitatory systems are dependent on the NMDA receptor (Larcher et al., 1998). It seems that blockade of NMDA receptors may reveal the full analgesic potency of an opioid. These results could explain the clinical potentiation of morphine analgesia by low doses of ketamine (Javery et al., 1996; Stubhaug et al., 1997).

Opioids also stimulate the central release of anti-opioid peptides, which then attenuate the antinociceptive effects of opioids (Cesselin, 1995). It has been reported that in the anaesthetised rat, cholecystokinin alone facilitates C-fibre responses and that when associated with DAGO (a μ-opioid receptor agonist), it increases the initial excitatory effect and reduces the inhibitory effect of the opioid (Magnusson et al., 1990). Cholecystokinin facilitated the response to noxious stimuli and partially masked the analgesic effect of DAGO. This result supports the notion that adaptive mechanisms involving opioid-activated pain facilitatory systems may participate in a homeostatic system leading to a reduction of the analgesic effects of opioids (Rothman, 1992).

Development of acute tolerance has been observed in animals (Kissin et al., 1991; Larcher et al., 1998) and human volunteers with remifentanil (Vinick and Kissin, 1998). Acute tolerance and the quick offset of action of remifentanil could explain hyperalgesia during the immediate postoperative period. It is possible that the facilitation of the C-fibre reflex in our model reflects stimulation by sufentanil, systems of which facilitate nociception and result in a competition between inhibitory and excitatory effects. At its lowest concentration, the stimulating effects of sufentanil predominate whereas following the highest concentrations, it elicits mainly inhibitory effects while the excitatory effects are masked.

In conclusion, the present methodology for recording and analysing a C-fibre evoked reflex represents an experimental model with a high level of applicability to evaluating the effects of both opioid and non-opioid putative analgesic drugs. This is because the model provides an ethically acceptable preparation for the quantification of the spinal transmission of nociceptive information at both threshold and suprathreshold levels. Our results show that different i.v. and i.t. doses of sufentanil result in a dose-dependent depression of a nociceptive flexion reflex by a direct spinal effect. At the highest stimulus intensities, the small shift to the right produced by sufentanil reflects a

higher intrinsic efficacy. The ED_{50} ratios for i.v. to i.t. sufentanil were near 1, probably because sufentanil is highly soluble in lipids. Sufentanil-induced facilitations relate to supraspinal actions on motor controls and/or on the descending modulation of nociceptive transmission.

Acknowledgements

The authors thank Dr S.W. Cadden for advice in he preparation and correction of the manuscript. This work was supported by l'Institut National de la Santé et de la Recherche Médicale (INSERM), by Institut UPSA de la Douleur and by Direction Régionale de la Recherche Clinique de l'Assistance-Publique Hôpitaux de Paris (CRC 96028).

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