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Effects of clonidine on a C-fibre reflex in the rat

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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 anaesthetized rats. The temporal evolution of the response was studied using a constant stimulus intensity (3 × threshold) and recruitment curves were built by varying stimulus intensity from 0 to 7 × threshold. The intravenous administration of 0.02–0.2 mg/kg clonidine resulted in a dose-dependent depression of the C-fibre reflex. The α_2-adrenoceptor antagonist idazoxan completely prevented this depressive effect of clonidine. The effects of clonidine on the C-fibre reflex elicited by a wide range of stimulus intensities were investigated using recruitment curves: following 0.16 mg/kg clonidine, a dramatic shift of the recruitment curve to the right was seen with both an increase in the threshold and a decrease in the slope. Clonidine also produced a dose-dependent increase in blood pressure, but this was not correlated with the depression of the nociceptive reflex.

Keywords: C-fiber; Reflex; Pain; Clonidine; Blood pressure; (Rat)

1. Introduction

The α_2-adrenoceptor agonists (e.g. xylazine and detomidine) are commonly used in veterinary medicine for their sedative, analgesic and myorelaxant properties (Clarke and Hall, 1990). Clonidine is also used in humans for reducing the anaesthetic requirements and for improving cardiovascular and, more generally, autonomic stability during surgery (for a review, see Maze and Tranquilli, 1991; Aantaa and Scheinin, 1993).

The analgesic properties of adrenoceptor agonists were first described by Weber (1904). Schmitt et al. (1974) demonstrated a profound analgesic activity of α-sympathomimetic compounds administered in the ventricles. The participation of noradrenergic neurones in modulating nociception is supported by pharmacological experiments using a number of tests, which have demonstrated the potent antinociceptive effects of the systemic (Paalzow, 1974; Fielding et al., 1978; Dennis et al., 1980; Chen et al., 1987; Clarke et al., 1988) or intrathecal administration of noradrenergic agonists (Yaksh, 1985; Kuraishi et al., 1985; Barasi and Clathworthy, 1987; Howe et al., 1987; Wiesenfeld-Hallin, 1987; Danzebrink and Gebhart, 1990; Nagasaka and Yaksh, 1990; Takano et al., 1992; Takano and Yaksh, 1992; Kanui et al., 1993; Kyles et al., 1993). More recently, the search for an effective alternative and/or adjunct to opioid therapy in the management of pain, brought about the central administration of α_2-adrenoceptor agonists (for ref. see Aantaa and Scheinin, 1993). Although there is a great deal of data to support a synergistic interaction between α_2-adrenoceptor agonists and opioids in the spinal cord (Yaksh and Reddy, 1981; Sullivan et al., 1987; Wilcox et al., 1987; Drasner and Fields, 1988; Ossipov et al., 1989, 1990; Monasky et al., 1990), there is also evidence that there may be a significant spinal component which is independent of opioid receptor mechanisms (Solomon and Gebhart, 1988; Ossipov et al., 1989). Cross-tolerance between α_2-adrenoceptors and μ-opioid receptors (Stevens et al., 1988) but not between α_2-adrenoceptors and δ-opioid receptors (Kalso et al., 1993) has also been reported.

We have described a reliable and highly sensitive electrophysiological method for the pharmacological study of the spinal transmission of nociceptive signals in rats, based on the electromyographic recording of a flexion reflex evoked by C-fibre activation (Strimbu-Gozariu et al., 1993).

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This reflex, elicited by electrical stimulation within the territory of the sural nerve and recorded in the ipsilateral biceps femoris muscle, is highly sensitive to intrathecal morphine. In addition, it provides information regarding the effect of a pharmacological agent on responses to both liminal and supraliminal levels of stimulation. We further validated the method by studying the effects of specific μ-, δ- and κ-opioid receptor antagonists against this spinal antinociceptive effect of morphine (Guirimand et al., 1994).

In the present experiments, we studied the effect of another putative analgesic drug, clonidine, a specific α_2-adrenoceptor agonist, administered systemically. The hypothesis that the effect of clonidine on the reflex was mediated through α_2-adrenoceptors was tested by using idazoxan, a selective antagonist of α_2-adrenoceptors. Blood pressure was recorded in parallel with the electromyographic responses, in order to investigate any correlation between these two parameters.

2. Materials and methods

2.1. General procedure

The general procedure was similar to that described previously (Strimbu-Gozariu et al., 1993). The experiments were carried out on male Sprague-Dawley rats (5–6 animals/group), weighing 325–375 g. During the surgical procedures, the animals were deeply anaesthetized with 2.5% halothane in a nitrous oxide/oxygen mixture (2/3 : 1/3). The rats were artificially ventilated via a tracheal cannula. Intraarterial (i.a.) and intravenous (i.v.) catheters were inserted into the common carotid artery and jugular vein, respectively. After surgery, the concentration of halothane was lowered to 1.1% in 100% oxygen. The arterial blood pressure was continuously monitored via the i.a. catheter which was connected via a transducer to a chart recorder. The i.a. cannula was filled with heparinized (25 000 U.I./500 ml) saline. The heart rate was also monitored. Ventilation and anaesthesia were controlled continuously (respiratory rate: 50 counts/min; end-tidal CO_2: 3.2–3.5%; halothane level: 1.1%) with the aid of a capnometer (capnomac II, Datex Instruments, Helsinki, Finland). The body temperature was maintained at 37 ± 0.5°C, by means of a homeothermic blanket system.

2.2. Electrophysiological methods

These methods have been described previously (Strimbu-Gozariu et al., 1993; Falinower et al., 1994). Briefly, electrophysiological recordings were made from the ipsilateral biceps femoris muscle, of C-fibre-evoked reflex activity elicited by electrical stimulation of the sural nerve receptive field. A pair of non-insulated Pt-Ir needle electrodes were inserted subcutaneously in the medial part of the fourth and lateral part of the fifth toe. Electromyographic (EMG) responses were recorded via another pair of non-insulated Pt-Ir needles, inserted 0.5 cm through the skin into the biceps femoris muscle.

The electrical stimuli were single, square-wave shocks of 2 ms duration and were delivered once every 6 s (0.17 Hz) from a constant-current stimulator. The stimulus intensities and the EMG responses were fed to an oscilloscope for continuous monitoring and to a computerised system for on-line digitisation. The digitised EMG responses were full-wave rectified and the C-fibre-evoked responses integrated within a time window of 100–450 ms after the stimulus onset. 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 μV × ms and the current intensities in mA). When investigating the recruitment curves, the stimuli were applied at increasing intensities from 0 mA to 7 × the threshold of the reflex.

2.3. Pharmacological procedures

Usually 20–30 min after the end of the surgical preparation and decrease in the level of anaesthesia, the application of 15 mA stimuli to the sural nerve resulted in stable supramaximal reflex responses with minimal spontaneous fluctuations. This was the preliminary, sine qua non, finding before the start of the subsequent procedures. By increasing the intensity of the stimulus, the reflex responses increased monotonically and reached a plateau at high intensities. The threshold of the C-fibre-evoked response was determined as the intersection of the polymodal regression curve and the abscissa. A constant level of stimulation (3 × threshold) was then employed.

A preliminary pilot study was made with increasing doses of clonidine, in order to obtain an overview of the time course of the effect on the reflex. Only the highest dose injected (0.16 mg/kg) had a noteworthy, long-lasting inhibitory effect on the C-fibre reflex, which permitted a more complex analysis by the use of the recruitment curve paradigm. We therefore undertook two series of experiments:

2.3.1. Effects of i.v. clonidine on the C-fibre reflex in a constant current paradigm

In a first series of experiments, a constant level of stimulation (3 × threshold) was applied and the reflex responses and mean arterial blood pressures were recorded. After a 10 min control period, i.v. clonidine hydrochloride was administered at three different doses, namely 0.016, 0.05 and 0.16 mg/kg and recordings were made for 60–150 min, depending on the duration of the pharmacological effects. Saline (0.4 ml) was injected in controls.

In an additional group, following the 10 min control period, idazoxan (0.1 mg/kg i.v.) followed 10 min later by clonidine (0.016 mg/kg i.v.) were administered. A pilot study had shown that this was the highest dose of idazoxan

with no significant depressive effect on the C-fibre reflex, when injected alone.

2.3.2. Effects of i.v. clonidine on the C-fibre reflex in a recruitment curve paradigm

In the second series of experiments, a control recruitment curve was built and clonidine (0.16 mg/kg i.v.) was injected 10 min later. Recruitment curves were then built at 15 min intervals for 240 min.

2.4. Processing of data

2.4.1. Analysis of data from the constant stimulation paradigm

Each individual EMG response was expressed as a percentage of the mean control value, calculated during the 10 min period that preceded the injection of clonidine. The results for individual animals were expressed ultimately as means of 50 successive responses, corresponding to 5 min of the procedure. The mean values were used for representing and analysing the time course for each dose of clonidine. For each dose, one-way analysis of variance (ANOVA), followed by Fisher posteriori least-significant difference (PLSD) post-hoc tests were used.

In order to calculate the dose-response relationship, the effects of clonidine were considered over a 40 min period. This period was chosen because it corresponded to a constant, maximal inhibition of the reflex following the highest (0.16 mg/kg) dose of clonidine (see Fig. 2C). Thus, for each rat, all individual responses were expressed as percentages of the control. The area under the curve (AUC) during the 40 min post-injection period was then calculated using the trapezoidal rule; the AUC was divided by the number of responses, namely 400, which corresponded to the 40 min of recording. This gave for each individual animal the mean effect of the injection in percentage terms with respect to the control and a dose-response curve was built on a semi-logarithmic plot.

One-way ANOVA and post-hoc tests (Fisher PLSD) were used to compare these AUC, and this was followed by linear regression analysis.

In parallel to the C-fibre reflex recordings, the mean arterial blood pressure was determined for every minute of the experiment and then expressed as a percentage of the basal blood pressure value. The results for individual animals were finally expressed as means of 5 successive values, corresponding to 5 min of the procedure. For each dose of clonidine, the time courses of the effects on the blood pressure were calculated by plotting individual results.

The significance of the effects of clonidine in time was tested by one-way ANOVA, followed by Fisher (PLSD) post-hoc tests.

The effect of idazoxan alone was determined by expressing each individual EMG response as a percentage of the mean control value calculated during the 10 min that preceded its injection, and tested by one-way ANOVA, followed by Fisher (PLSD) post-hoc tests.

The response to clonidine (0.016 mg/kg) following the antagonist, was expressed in each individual case as a percentage of the mean control value, calculated during the 5 min preceding the injection of clonidine. The results for individual animals were expressed finally as means of 50 successive responses, corresponding to 5 min of the procedure. The time courses of the effects were calculated by plotting individual results. A two-way ANOVA followed by Fisher (PLSD) post-hoc tests were used to compare this time course to that produced by clonidine alone.

2.4.2. Analysis of data from the recruitment curve paradigm

In order to build the recruitment curves, individual EMG responses were expressed as percentages of the maximal control C-fibre reflex response which was generally recorded at an intensity of 7 × reflex threshold. Stimulus intensities were expressed as multiples of the threshold calculated during the control period. More than 15 points were used to build each recruitment curve. However, to simplify the processing of data, only 9 points, namely 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7 × threshold intensity, were considered. When one of these intensities had not actually been applied during the experiment, the response was estimated by linear interpolation between the nearest two points. Such interpolations never involved intensities greater than 2 mA.

The areas under the recruitment curves corresponding to the control period (AURC control) and to each 15 min following the drug administration (AURC post-clonidine) were calculated in each case and for each animal, by using trapezoidal approximation. Note that the absolute values of the integrals corresponding to the C-fibre responses (in mV × ms) were considered. One-way ANOVA, followed by Fisher (PLSD) post-hoc tests were used to compare these AURC.

The thresholds of the C-fibre responses (in mA) and the slopes of the fitted curves (in mV × ms/mA) corresponding to the linear part of the recruitment curves were calculated and the respective means ± S.E.M. were then considered. One-way ANOVA, followed by Fisher (PLSD) post-hoc tests were used to compare these thresholds and slopes.

Data were expressed as means ± S.E.M. Results were considered significant at $P < 0.05$.

3. Results

3.1. General characteristics of the reflex responses

As illustrated with a control response in Fig. 1, electrical stimulation within the territory of the sural nerve elicited a two-component reflex response in the ipsilateral biceps femoris muscle. The first component with a short

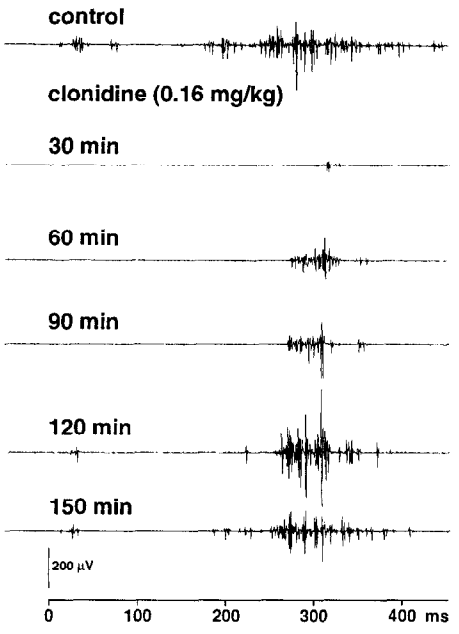

Fig. 1. Individual example of reflex responses to C-fibre activation. The recordings were made from the ipsilateral biceps femoris muscle, after stimulation of the sural nerve territory at a constant stimulus intensity (3 × the threshold of the control). The control was recorded 10 min before clonidine administration (0.16 mg/kg; i.v.); the time following its injection is indicated above each recording. Note that 30 min after clonidine injection, the A and C-fibre responses were strongly depressed; a progressive recovery of the responses to magnitudes close to the control level was seen at 150 min.

latency (10–20 ms), short duration (< 50 ms) and low threshold (0.5–2 mA range), has been shown to be triggered by activity in myelinated fibres (Falinower et al., 1994), and was not analyzed here. By contrast, we carefully considered at a quantitative level, the second component which exhibited a longer latency (mean: 160 ± 4 ms at 3 × threshold intensity), longer duration (mean: 289 ± 5 ms at 3 × threshold intensity) and higher threshold (mean: 5.4 ± 0.4 mA). Such a response results from activation of unmyelinated cutaneous afferent C-fibres (Strimbu-Gozariu et al., 1993; Falinower et al., 1994), and we refer to it as the C-fibre reflex.

Two series of experiments were performed. The first aimed to determine the effects of increasing doses of i.v. clonidine on both the blood pressure and the C-fibre reflex elicited by a constant level of stimulation. In order to perform a more complete analysis of the effects of the most active dose of clonidine, a second series of experiments was performed, with increasing intensities of the stimulus. Injections of saline in a control group were ineffective.

3.2. Effects of i.v. clonidine on the C-fibre reflex in a constant stimulation paradigm

Fig. 1 shows individual examples of recordings of the electromyographic response elicited by stimulating the sural nerve at a constant stimulation intensity, namely 3 × the control threshold. The individual examples were selected from a constant stimulation experiment, 10 min before (control) and each half hour following the intravenous administration of 0.16 mg/kg clonidine. Note the strong depression of all components of the reflex which were virtually absent for half an hour and recovered slowly

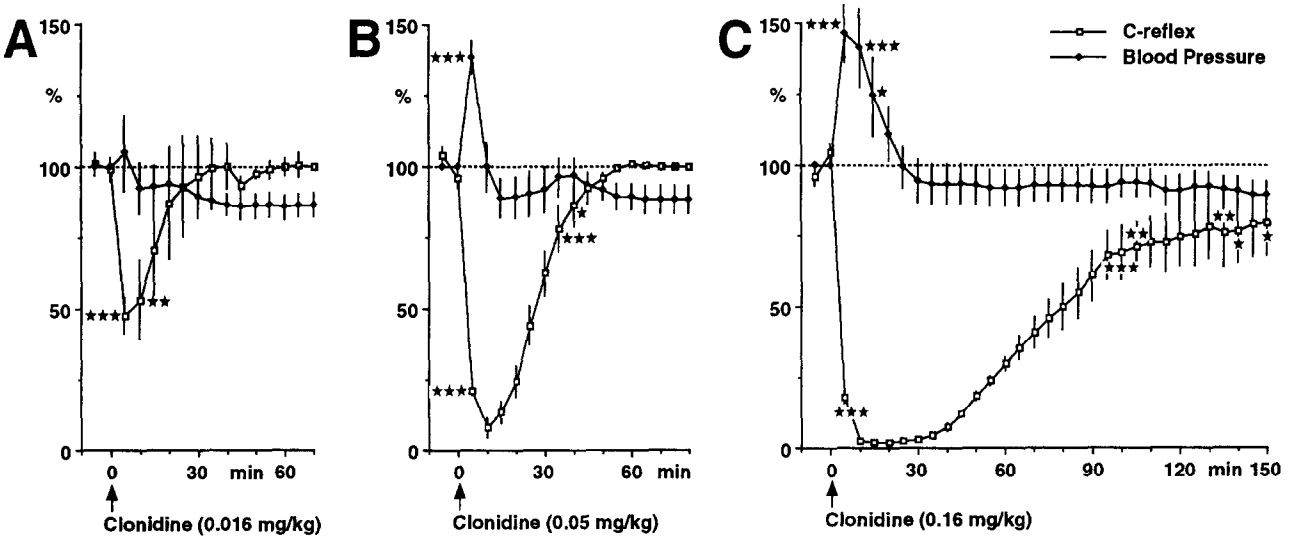

Fig. 2. Overall results, showing the time courses of the effects of 0.016 (A); 0.05 (B) and 0.16 mg/kg (C) of i.v. clonidine on the C-fibre reflex (white symbols) and on blood pressure (black symbols). In each case, clonidine was injected (arrows) after a 10 min control period. Ordinate: C-fibre-evoked EMG responses and blood pressure, expressed as percentages of the mean control values calculated during the 5 min that preceded clonidine administration. The baseline blood pressure was 91.7 ± 3.2 mm Hg. Stars represent a significant decrease in the reflex or increase in blood pressure as compared to the 5 min period preceding the clonidine injection. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. All points between two identical symbols have the same level of significance (not shown for clarity of presentation).

towards control values in the 150 min following clonidine administration.

The overall results are presented in Fig. 2, which illustrates the time courses of the effects for three different doses of intravenous clonidine on both the C-fibre reflex and blood pressure. At a dose of 0.016 mg/kg (Fig. 2A), clonidine had no significant effect on blood pressure, but elicited a partial and short-lasting depression of the C fibre reflex (50% range) which was significant for 10 min and had recovered completely after 30 min. At a dose of 0.05 mg/kg (Fig. 2B), clonidine induced a significant (40% range) but short-lasting (5 min) increase in the blood pressure, and a large depression of the C-fibre reflex, which was rapid in onset (90% range at 10 min), significant for 40 min and had recovered completely by 60 min. At a dose of 0.16 mg/kg (Fig. 2C), clonidine elicited an immediate increase in blood pressure, which peaked at around 40% within 10 min, was significant for 15 min and had recovered by 20 min. The corresponding picture for the C-fibre reflex was very different. An almost complete blockade of the reflex was seen for half an hour, followed by a very slow recovery towards the control level of response. Two and a half hours after the drug administration, recovery had not been achieved, with the C-fibre reflex only 75% of the control value.

Regarding heart rate, clonidine elicited bradycardia that did not reach statistical significance. Bradycardia occurred mainly during clonidine administration, was maximum at 1 min (e.g. 11–15% reduction of heart rate following 0.16 mg/kg), with a rapid recovery towards a stable level within 10–15 min post-administration; however, this level was slightly lower than controls till the end of the experiment (e.g. 6–8% reduction of heart rate following 0.16 mg/kg during 150 min post-administration).

Fig. 3A shows the dose-response relationship for the effect of clonidine on the C-fibre reflex, expressed in terms of the mean value of the response calculated during the 40 min period following the drug administration and expressed as a percentage of the controls. Note the linear relationship between the mean size of response and the log of the dose. In the 0.016–0.16 mg/kg range, all doses of clonidine had a significant effect on the reflex, as compared to saline which was ineffective (mean 'depression' of the reflex during the 40 min: 2.07 ± 2.04%). The ED_{50} for clonidine was 0.038 mg/kg.

The effects of the α_2-adrenoceptor antagonist, idazoxan, were investigated in a further series of experiments. In a pilot study, we observed that the antagonist itself could trigger depressive effects on the C-fibre reflex, for instance following the administration of 0.5 or 1 mg/kg. It was found that 0.1 mg/kg idazoxan was the highest dose with no significant depressive effect on the C-fibre reflex. Administered 10 min before clonidine, such a low dose completely prevented the depressive effects (Fig. 3B).

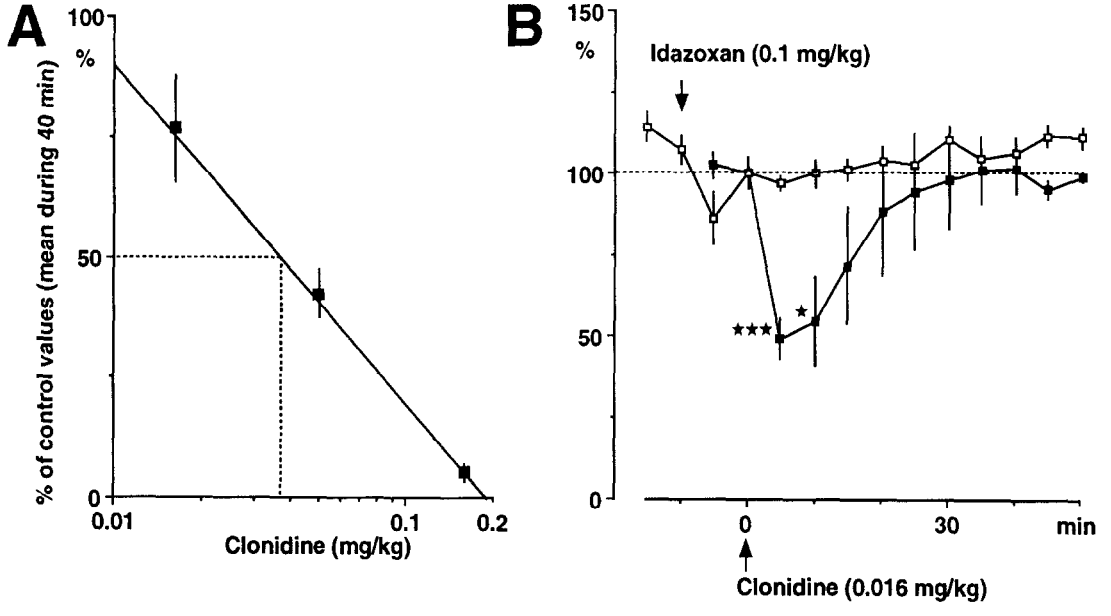

Fig. 3. (A) Dose-response curve obtained with a constant stimulus intensity (3 × threshold of reflex during the control period). The curve was built by plotting the mean value of the responses calculated in terms of percentage of controls, during the 40 min following clonidine administration (ordinate), against the dose (mg/kg) on a logarithmic scale (abscissa). As compared to saline (not shown), a significant depression of the reflex occurred in the 0.016–0.16 mg/kg range ($F(4,20) = 45.3$; $P < 0.0001$). Note the linear correlation between the mean percentage of depression and the log of the dose ($y = -71.35 \log x - 51.3$; $r_{13} = 0.89$; $P < 0.0001$). The ED_{50} (dotted lines) was 0.038 mg/kg. (B) Overall results showing the time courses of the effects on the C-fibre-evoked reflex of clonidine (0.016 mg/kg) alone (white squares) and of idazoxan (0.1 mg/kg), followed by clonidine (0.016 mg/kg) (black squares). In the first case, clonidine was injected after a 10 min control period (lower arrow). In the second case, idazoxan was injected after 10 min of recording (upper arrow) and clonidine was injected 10 min later (lower arrow). The 5 min preceding clonidine injection were considered as a control period in both cases and the effects of the drugs were expressed as percentages of these control values (ordinate). The two time courses differ significantly during the first 10 min following clonidine injection ($F(1,8) = 4.13$; $P < 0.05$). Note also a slight but not significant depression of the reflex following idazoxan.

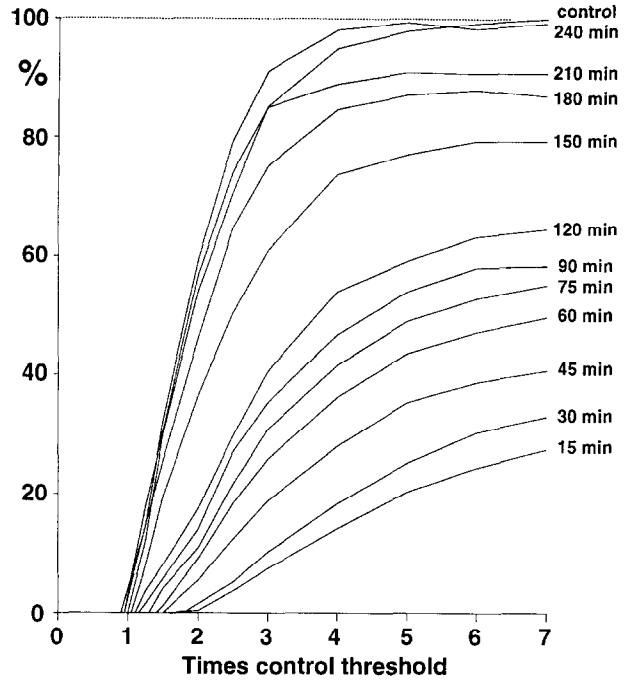

Fig. 4. Overall results showing the effects of 0.16 mg/kg of i.v. clonidine upon the C-fibre reflex to a wide range of stimuli. The recruitment curves were made 10 min before (control) and at various moments indicated on the right side of each curve following clonidine administration. Abscissa: current intensities, expressed as times the threshold of the control C-fibre reflex responses. Ordinate: integrals of the C-fibre responses during a 100–450 ms time window, expressed in each case as a percentage of the maximum C-fibre control values, generally recorded at an intensity of 7 × the threshold. S.E.M. ≤ 13.2% are not shown for clarity of presentation.

3.3. Effects of i.v. clonidine on the C-fibre reflex in a recruitment curve paradigm

These experiments were undertaken in order to study the effects of clonidine on the C-fibre reflex elicited by a wide range of stimulus intensities. The highest, 0.16 mg/kg, single dose of clonidine was chosen because, as described above, it produced depressive effects during a long enough period to allow recruitment curves to be built.

In each individual case, 16 recruitment curves for the C-fibre reflex were made over 250 min, namely 10 min before (control) and every 15 min following clonidine administration. Each individual recruitment curve was built as follows: as with the constant stimulation paradigm, the electromyographic C-fibre responses were integrated within a 100–450 ms window, but each individual value was expressed in percentage terms with respect to the maximum value reached in the control situation; these individual values were plotted on the ordinate of a graph against an abscissa of stimulus intensity expressed as multiples of the control threshold.

Fig. 4 summarises the overall results obtained with such an experimental protocol. The mean control curve was steep, linear within the 1–3 × threshold range, and plateaued within the 4–7 × threshold range. Fifteen minutes following 0.16 mg/kg clonidine, a dramatic shift of the curve to the right was seen with both an increase in the threshold and a decrease in the slope. The highest stimulus intensities (7 × threshold) were followed by a response reduced by three quarters. As illustrated in Fig. 4 from

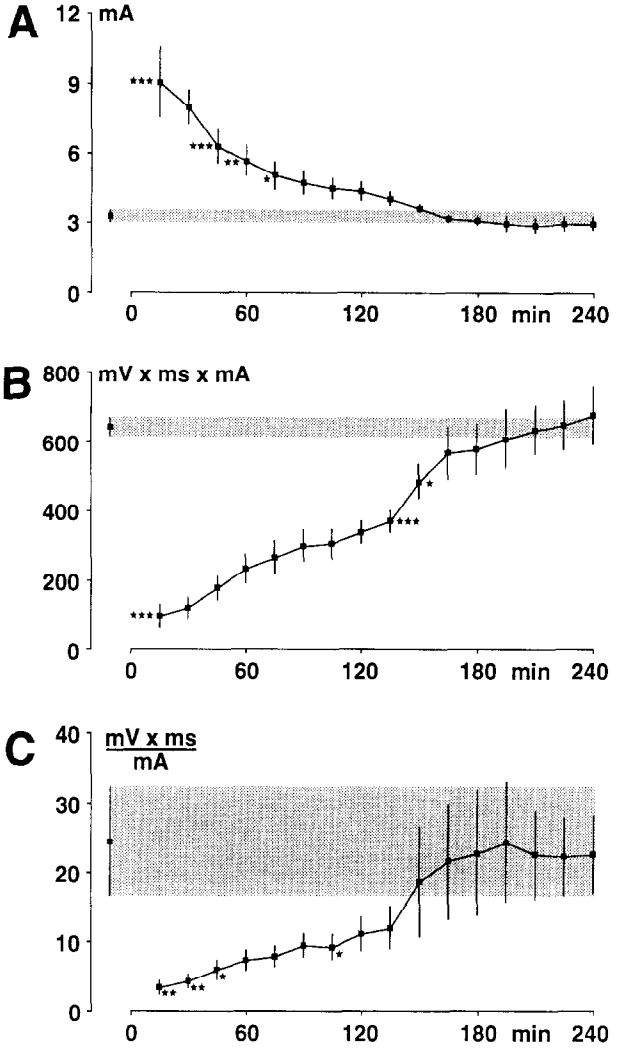

Fig. 5. Overall results showing the time courses of the thresholds of the C-fibre responses (A), the area under the recruitment curves (B) and the slopes of the recruitment curves (C), determined every 15 min following 0.16 mg/kg of i.v. clonidine during 240 min of recordings (abscissa). The shaded areas correspond to the mean control values ± S.E.M. calculated 10 min before clonidine administration. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$, as compared to controls (same significance between two identical symbols are not shown for clarity of presentation). (A) Threshold of the C-fibre reflex. Ordinate: current intensity (mA). In each individual case, the threshold was determined as the intersection of the linear part of the recruitment curve with the abscissa. (B) Areas under the recruitment curves (AURC). In each individual case, the AURC was calculated in the 1–7 × control threshold range. Ordinate: AURC in mV × ms × mA. (C) Slopes of the recruitment curves. In each individual case, the slopes of the recruitment curves were calculated as being those of the fitted curves. Ordinate: slopes in (mV × ms)/mA.

bottom to top, the recruitment curve progressively recovered within 2.5 h, with the last curve being virtually identical to the control.

More details regarding this experimental paradigm are provided in Fig. 5. The overall data regarding the threshold are shown in Fig. 5A. In each individual case, the threshold was determined as the intersection of the linear part of the recruitment curve and the abscissa. Note the rapid and abrupt increase in the threshold following clonidine injection and the progressive recovery within 2 h. Fig. 5B and C show the corresponding evolution of both the area under the recruitment curve and the slope of the linear part of these curves. Following a fall in the area under the recruitment curve immediately after clonidine injection, a progressive recovery occurred, with the values being significantly smaller than the control for 150 min (Fig. 5B). A comparable picture was seen with respect to the slope of the recruitment curves (Fig. 5C) with an abrupt decrease following clonidine injection, a progressive recovery for 130 min and a sudden change at 135 min.

Note that in the period from 15 to 75 min, both the threshold and the slope of the recruitment curve were significantly modified. In the period from 75 to 105 min, although the threshold was similar to that of the control, the slopes continued to differ significantly, indicating an effect of clonidine at suprathreshold stimulus intensities.

4. Discussion

The stimulation of the sural nerve elicited a two-component reflex in the flexor muscles of the ipsilateral hindlimb, the first and the second component being elicited by activation of myelinated and unmyelinated afferent fibres, respectively (Falinower et al., 1994). As discussed elsewhere, convergent neurones in the lumbar spinal cord are part of the circuitry involved in the polysynaptic C-fibre-evoked reflex (Schouenborg and Sjölund, 1983; Falinower et al., 1994). The validation of this reflex as a sensitive tool for studying the pharmacology of nociception was provided by demonstrating its depression following very low doses of intrathecal morphine (Strimbu-Gozariu et al., 1993).

In the present study, clonidine exhibited a dose-dependent depressive effect on the C-fibre reflex in the constant stimulation intensity paradigm. Idazoxan completely prevented the effect of clonidine on the C-fibre reflex, confirming the role of central α_2-adrenoceptors in our experiments. A complete blockade of the EMG responses elicited by activation of C-fibres, occurred following 0.16 mg/kg clonidine. Using the same model, a comparable effect was obtained by Guirimand et al. (1995) following 10 mg/kg of morphine chlorhydrate. Our results are in agreement with previous reports in animals where different nociceptive tests were used (Fielding et al., 1978; Spaulding et al., 1979a). These studies revealed that intravenous clonidine has a higher potency than intravenous morphine in the 10–60 × molar range. In addition to the fact that they obviously act by different mechanisms, the higher efficacy of clonidine following systemic administration could be explained partly by its high liposolubility and predominant distribution in the central nervous system, as compared to the hydrophilic morphine. Thus, following intravenous injection, lipid soluble drugs are initially concentrated in, then redistributed away from neural tissue, producing a brief, but intense effect (Eisenach et al., 1987).

In the recruitment curves paradigm, the effects of clonidine on the C-fibre reflex elicited by a wide range of stimulus intensities were studied. The highest dose of clonidine (0.16 mg/kg) was chosen because the duration of the depressive effects was long enough to permit recruitment curves to be built. Fifteen minutes following 0.16 mg/kg clonidine, a dramatic shift of the mean curve to the right was seen, with both an increase in the threshold and a decrease in the slope. The mean recruitment curve progressively recovered over a period of 2.5 h. Comparable effects were noticed when high doses of i.v. morphine (6–10 mg/kg range) were administered and these are reminiscent of those reported following intrathecal administration of low doses of morphine (Strimbu-Gozariu et al., 1993). It was suggested that these effects probably resulted from a direct spinal effect of opioids.

By studying the effect of α_2-adrenoceptor agonists administered by various routes on different nociceptive tests in several preparations, many authors have found a significant antinociceptive effect (Yaksh, 1985; Takano and Yaksh, 1992; Kanui et al., 1993; Nagasaka and Yaksh, 1990; Wiesenfeld-Hallin, 1987; Rawlow and Gorka, 1986; Rawlow and King, 1991; Clarke et al., 1988; Schomburg and Steffens, 1988; Ossipov et al., 1990; Wilcox et al., 1987; Barasi and Clathworthy, 1987; Kuraishi et al., 1985; Spaulding et al., 1979a; Jones and Gebhart, 1986; Danzebrink and Gebhart, 1990, 1991; Pertovaara et al., 1990). Tail flick was the most commonly used test, but the increase in tail flick latency could be due partly to changes in skin temperature (Tjolsen et al., 1990). Recording a nociceptive spinal reflex elicited by electrical stimulation allowed such a complication to be avoided in the present experiments.

A central site for the antinociceptive effect of α_2-adrenoceptor agonists has been suggested because they are more potent following central than systemic administration (for ref. see Yaksh, 1985). The question of whether such antinociceptive effects of α_2-adrenoceptor agonists are mediated through spinal or supraspinal mechanisms has been a matter of debate. There is evidence for the antinociceptive effects of α_2-adrenoceptor agonists being exerted, either directly or indirectly, at a spinal level. The major site where noradrenaline acts by inhibiting the transmission of nociceptive signals appears to be the dorsal horn of the spinal cord since many noradrenaline axon terminals are found at this level (for ref. see Proudfit, 1992). Spinal

α_2-adrenoceptors are located on nociceptive primary afferent terminals and on small interneurones in the substantia gelatinosa (for ref. see Headley, 1992). In vivo, electrophoretic or intrathecal administration of α_2-adrenoceptor agonists inhibited the nociceptive-evoked responses of dorsal horn convergent neurones. Such a depression appeared to be mediated through both presynaptic and postsynaptic mechanisms. These conclusions were confirmed in in vitro experiments (for ref. see Fleetwood-Walker, 1992; Headley, 1992; Proudfit, 1992).

However, the innervation of the spinal cord by noradrenergic neurones is extensive and includes the superficial dorsal horn, the intermediate zone and the ventral horn (for ref. see Proudfit, 1992; Westlund, 1992). Only pontine catecholamine cell groups, namely, locus coeruleus (A_6), locus subcoeruleus, the parabrachial nuclei, A_5 noradrenergic cell group, and Kölliker-Fuse nuclei, project to the spinal cord. The locus coeruleus/subcoeruleus is believed to play the major role in antinociception. Indeed, stimulation of these cell groups produces antinociception which is blocked by intrathecal injection of α_2- (but not α_1-) adrenoceptor antagonists (for ref. see Proudfit, 1992).

With respect to the spinal projections of locus coeruleus/subcoeruleus, clear differences between rat sub-strains, namely Sasco and Harlan Sprague-Dawley, have been reported. In the former, they innervate the dorsal horn and modulate nociception, while in the latter they innervate the ventral horn. In addition, other brainstem neurones are involved in the noradrenergic modulation of nociception; for example, neurones in the periaqueductal grey and the ventromedial medulla project to and activate ponto-spinal noradrenergic neurones (for ref. see Proudfit, 1992).

The question arises as to whether the effects of clonidine were on the sensory and/or the motor part of the reflex arc. Indeed α_2-adrenoceptors have also been found around motoneurones in the ventral horn of the spinal cord and axo-somatic contacts between catecholamine fibres and motoneurones have been described in the lumbo-sacral spinal cord of the rat (for ref. see Headley, 1992; Proudfit, 1992). When administered in the ventral horn, noradrenaline can produce a significant hyperpolarization of motor neurones and a depression of interneurones in the vicinity of the motor neurone pool (Engberg and Ryall, 1966; Weight and Salmoiraghi, 1966; Jordan et al., 1977). However, excitation after focal administration has been observed and appears in accord with the observation that noradrenaline can produce a facilitation of C-fibre-evoked ventral root reflexes (Takagi et al., 1955; White and Neuman, 1980). It appears possible that the depressive effects are mediated by α_2-adrenoceptors, whereas the facilitatory effects are mediated through α_1-adrenoceptors (for ref. see Yaksh, 1985). On the basis of agonist and antagonist structure-activity relationships, it was demonstrated that a significant antinociceptive effect can be produced by the occupation of α_2-adrenoceptors by analgesic doses of α_2-adrenoceptor agonists in the absence of any apparent effects on motor function. Intrathecal noradrenaline or other α_2-adrenoceptor agonists elicited clear signs of hindlimb flaccidity only at doses higher than those required to block nociceptive responses (for ref. see Yaksh, 1985). Nevertheless, we cannot exclude that at least part of the effects of clonidine described herein were related to a depressive effect on the motoneuronal pool which elicits the C-fibre reflex. Preliminary experiments presently in progress in the laboratory suggest such a possibility, at least following the higher doses of clonidine tested.

The antinociceptive effect of systemic α_2-adrenoceptor agonists was reported to be present following spinalisation and absent following central administration (Spaulding et al., 1979b). Thus, clonidine whether microinjected into the ventrolateral periaqueductal grey, the locus coeruleus or the lateral reticular nucleus in the caudal medulla did not produce antinociceptive effects in any of three nociceptive tests employed, namely tail-flick, hot-plate and tail-shock vocalisation (Ossipov and Gebhart, 1983, 1986; Ossipov et al., 1984). In other experiments, however, where hindlimb flexor reflexes were used as nociceptive tests, systemic clonidine or other α_2-adrenoceptor agonists (tizanidine) suppressed reflex responses in intact but not in spinal animals, suggesting a bulbo-spinal mechanism (Kehne et al., 1985; Chen et al., 1987). It was speculated that the depression of both polysynaptic and monosynaptic reflexes, following high doses of noradrenaline or α_2-adrenoceptor agonists might also be caused by a removal, via α_2-autoadrenoceptors in locus coeruleus, of a descending noradrenaline facilitation tonically exerted on transmission in the ventral horn via α_1-adrenoceptors (Svensson et al., 1975; Fung and Barnes, 1987; Ono et al., 1988; Starke, 1987; Palmeri and Wiesendanger, 1990). This hypothesis is compatible with the further observation that microstimulation in locus coeruleus enhanced both mono- and polysynaptic reflexes, with such a facilitation being mediated by α_1-adrenoceptors at the spinal cord level (Chan et al., 1986; Fung and Barnes, 1987; Palmeri and Wiesendanger, 1990). α_2-Adrenoceptor agonists whether administered systemically or micro-electrophoretically, have been reported to decrease locus coeruleus neuronal activity through hyperpolarisation of the neuronal membrane of locus coeruleus neurones (Aghajanian and Van der Maelen, 1982).

Interestingly, Sullivan et al. (1987) studied the effect of intrathecal clonidine on electrically evoked C-fibre responses of convergent neurones in the dorsal horn of halothane (0.7–1%)/N_2O-anaesthetized rats; they reported a dose-dependent depressive effect of clonidine with a maximum of 52% following 150 μg clonidine. The difference with our results (ED_{50} = 45 μg, i.v.) could be due to several factors: (1) we recorded a global response to C-fibre activation; (2) the effects we reported could be the result of both a spinal and a supraspinal effect of systemic clonidine; (3) different surgical conditions could explain a

greater sensitivity of our test (see Strimbu-Gozariu et al., 1993); (4) some of our results could be explained by effects of clonidine on both the dorsal and the ventral horn of the spinal cord.

Clonidine, an imidazole compound, is a selective agonist for α_2-adrenoceptors, with a ratio 200:1 ($\alpha_2:\alpha_1$) (Hayashi and Maze, 1993). The effect of clonidine on the C-fibre reflex appeared to be mediated specifically through α_2-adrenoceptors since idazoxan completely prevented its effect. Idazoxan has a high selectivity for α_2-adrenoceptors compared to α_1-adrenoceptors (Raymon et al., 1992). Differences have been described in the rank order of potency of α_2-adrenoceptor antagonists in rodent versus non-rodent species; this is probably due to the heterogeneity of α_2-adrenoceptors (for ref. see Wilson et al., 1991). In our study, idazoxan was used since it shows a higher affinity than yohimbine for α_2-adrenoceptors in rodents. Its short-lasting inhibitory effect on the reflex might be due to some agonist-like properties. Similar agonist-like effects were reported with yohimbine in the hot plate and formalin tests in mice (Dennis et al., 1980; Kanui et al., 1993), but further investigations are required to elucidate these effects. One hundred μg/kg idazoxan, the highest dose with no significant depressive effect on the C-fibre reflex, was therefore used to prevent the effects of a low dose of clonidine (0.016 mg/kg), the latter being chosen in order to avoid saturation of α_2-adrenoceptors by the agonist. The blockade of the clonidine effects by idazoxan is in agreement with binding and pharmacological studies which stressed the fact that the analgesic effect of clonidine is mediated through α_{2A}-adrenoceptors, without excluding the involvement of α_{2C}-adrenoceptors (Uhlen et al., 1992), while idazoxan exhibits a great affinity for α_{2A}-adrenoceptors (Raymon et al., 1992). According to Millan (1992), it seems likely that α_{2A}-adrenoceptors are involved in antinociception, but it is not yet clear to what extent the α_{2B}- and α_{2C}-adrenoceptor subtypes are also involved in antinociceptive processes or the production of side-effects (Ruffolo et al., 1988). Since the rat spinal cord contains primarily α_{2A}- and α_{2C}-adrenoceptors (Uhlen et al., 1992), it is possible that these two receptors are responsible for spinal antinociception.

In any case, results similar to ours were reported following intrathecal idazoxan (100 μg) and clonidine (100 μg) with the hot plate test (Takano et al., 1992). Note that the molar ratio idazoxan/clonidine appeared to differ with the route of administration: following intrathecal injection, idazoxan could reverse the effect of clonidine more easily, probably for pharmacokinetic reasons.

From a general point of view, the antinociceptive effect of clonidine should be considered in relation to the sedative and anaesthetic-sparing effects of α_2-adrenoceptor agonists. Since our experiments were performed in halothane-anaesthetized rats, we cannot exclude interference between the two classes of pharmacological agents. Thus, Kehne et al. (1985) showed in intact non-anaesthetized rats, that i.p. clonidine elicited a depressive effect on a hindlimb flexor reflex with an $ED_{50} = 0.03–0.125$ mg/kg, depending on the intensity of stimulation and Ossipov et al. (1990) reported an $ED_{50} = 0.11$ mg/kg in the tail flick test, following i.v. clonidine. Therefore, the low ED_{50} (0.038 mg/kg, i.v.) in our experiments could be due not only to the high sensitivity of the method used but also to the fact that our animals were halothane-anaesthetized (1%). Such a role of anaesthesia could be inferred by considering experiments involving intrathecal administration of clonidine: Wilcox et al. (1987) and Nagasaka and Yaksh (1990) reported ED_{50} of 20 and 2.5 μg using the tail-flick test in non-anaesthetized and halothane (0.75%) anaesthetized rats, respectively. Thus, in the rat, the concomitant administration of clonidine allows a reduction in the minimum alveolar concentration (MAC) of halothane by 40–50% (Nagasaka and Yaksh, 1990; for ref. see also Aantaa and Scheinin, 1993). In humans, oral pretreatment with clonidine reduced isofluorane requirements by 40% (for ref. see Hayashi and Maze, 1993). Studies with selective antagonists have confirmed the involvement of central α_2-receptors in this effect (Maze and Tranquilli, 1991).

Clonidine induces sedation, a side-effect which might also contribute to the inhibition of spinal reflexes (Hayes et al., 1986; Butelman and Woods, 1993). The sedative property makes this class of drugs potentially useful for pre-anaesthetic medication (for ref. see Aantaa and Scheinin, 1993). In experiments performed in awake non-anaesthetized monkeys, a depressant effect of α_2-adrenoceptor agonists on polysynaptic reflexes was noticed in parallel with a general sedation and a striking decrease in spontaneous limb and eye movements (Corboz et al., 1991). These authors concluded that some of the motor changes induced by α_2-adrenoceptor agonists were expressed indirectly via ascending fibres from the noradrenergic locus coeruleus neurones to motor structures, like the motor cortex. Others have suggested that sedation occurs through direct activation of α_2-adrenoceptors in locus coeruleus (for ref. see Aantaa and Scheinin, 1993; Hayashi and Maze, 1993).

In our experiments, the depression of the nociceptive reflex was not correlated with changes in blood pressure. Although acute hypertension could depress nociceptive reflexes, the duration of the rise of blood pressure in our study, which was significant following high doses of clonidine, was much shorter than the duration of the depression of the reflex. Similarly, Schomburg and Steffens (1988) showed in cats that blood pressure was generally increased after i.v. injection of clonidine, but neither the extent, nor the time course of these rises were correlated with any effects on reflex pathways. The hypertension could be explained by a peripheral vasoconstrictive effect of clonidine, injected in bolus, by activation of post-synaptic α_1/α_2-adrenoceptors (for a review see Van Zwieten, 1990). It was also shown that α_2-adrenoceptors were extrajunctional, in contrast to the α_1-adrenoceptors which were

junctional (for ref. see Ruffolo and Hieble, 1994). The short hypertensive effect was followed by a long-lasting hypotensive effect – non-significant in our experiments – which is most probably due to activation of central α_2-adrenoceptors in the ponto-medullary region. It is generally accepted that clonidine stimulates central postsynaptic α_2-adrenoceptors of inhibitory neurones (possibly belonging to bulbospinal pathways), which in turn will lower the peripheral sympathetic activity and cause a hypotensive effect (for a review see Van Zwieten, 1990). The nucleus tractus solitarii and/or the lateral reticular nucleus in the ventrolateral medulla are considered as the main targets of the central α_2-adrenoceptors in this respect. Note that imidazole receptors have also been described in the rostral part of the ventro-lateral medulla and could also mediate these hypotensive effects. However, α_2-adrenoceptors are still the main target of clonidine-type drugs, whereas imidazole receptors may be considered as an auxiliary second target which might modulate the process triggered by α_2-adrenoceptor stimulation (for a review see Van Zwieten, 1990).

None of the clonidine doses studied here elicited significant hypotension, a side-effect often considered as a limiting factor for the clinical use of the drug. In humans, hypotension and bradycardia were produced by intrathecal clonidine. However, the hypotensive effect of clonidine was found to be decreased in non-hypertensive subjects. No change or an increase in blood pressure occurred following intravenous or at high doses, following intrathecal or epidural administration of clonidine (for ref. see Eisenach et al., 1987; Filos et al., 1994). All these effects were dependent on the plasma concentration of the drug. Systemically administered lipid soluble drugs, like clonidine, produced hypotension by acting on brainstem neurons. This action was counteracted by peripheral vasoconstriction at certain plasma concentrations of the drug. Intrathecally administered clonidine produced hypotension by two mechanisms: redistribution of the drug to brainstem sites of action and direct spinal inhibition of preganglionic sympathetic outflow (for ref. see Eisenach et al., 1987). These authors postulated that plasma concentrations of clonidine sufficiently high and sustained to trigger peripheral vasoconstriction, could prevent the hypotensive effects of the drug at central sites.

In our experiments, the depression of the nociceptive reflex was not correlated with the slight changes of heart rate. The bradycardia elicited by clonidine is a rather complex phenomenon. Thus, at least part of the clonidine-induced bradycardia is mediated by the same central receptors and is due to the same type of mechanisms that are involved in the hypotensive action. In addition, clonidine was shown to stimulate central α-adrenoceptors which enhance vagal reflex bradycardia. Peripheral mechanism might also have contributed to the bradycardia induced by the drug: this could be the result of an inhibition of the cardiac sympathetic tone via presynaptic α_2-adrenoceptors. Together, these mechanisms could explain the bradycardia seen during both hyper- and hypotension following i.v. clonidine (for rev. see Van Zwieten, 1990).

In summary, we have described a dose-dependent depression of a C-fibre reflex following the intravenous administration of clonidine in the 0.02–0.2 mg/kg range. These effects are mediated by α_2-adrenoceptors and are not related to changes in blood pressure or heart rate. Experiments are now in progress to define further the respective contributions to these effects of clonidine, from the sensory and/or motor parts of the reflex arc.

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