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Involvement of spinal dopamine receptors in mediation of the hypotensive and bradycardic effects of systemic quinpirole in anaesthetised rats

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Abstract

This study examined the involvement of spinal dopamine D_2 receptors in the cardiovascular effects induced by intravenous administration of the selective dopamine D_2 receptor agonist quinpirole, as has been previously reported for the hypotensive action of systemic bromocriptine. In normotensive pentobartitone-anaesthetised rats, intravenous injection of quinpirole (25 to 1000 μg/kg) decreased mean aortic pressure and heart rate in a dose-related manner. The intravenous (0.5 mg/kg) or intrathecal (40 μg/rat at T9–T10) pretreatment with domperidone, a dopamine D_2 receptor antagonist that does not cross the blood-brain barrier, significantly reduced the maximal hypotensive and bradycardic responses to intravenous quinpirole (1000 μg/kg). In contrast, the latter effects were fully abolished either by intravenous metoclopramide (5 mg/kg) or combined pretreatment with intravenous and intrathecal domperidone. In addition, when injected intrathecally at the T9–T10 level of the spinal cord, quinpirole (7.7 to 61.4 μg/rat) also produced dose-dependent depressor and bradycardic effects which could be blocked by intrathecal, but not intravenous, domperidone pretreatment. This suggests that, in anaesthetised normotensive rats, the hypotensive and bradycardic responses to intravenous quinpirole are fully mediated by dopamine D_2 receptors, some of which are located in the peripheral circulation and some of which are located within the spinal cord. The latter finding is novel, suggesting that partial spinal mediation may not be peculiar to bromocriptine, as was previously thought. Rather, partial spinal mediation may be common to most dopamine D_2 receptor agonists.

Keywords: Quinpirole; Cardiovascular effect; Intrathecal domperidone; Dopamine D_2 receptor, spinal; Spinal mediation, partial; Normotensive rat

1. Introduction

Quinpirole (LY171555), a highly selective dopamine D_2 receptor agonist (Tsuruta et al., 1981; Ruffolo and Shaar, 1983; Stoof and Kebabian, 1984; Seeman and Schaus, 1991), induces different effects on blood pressure and heart rate according to the experimental procedure (e.g., the use of anaesthesia and strain of animal). Thus, quinpirole was shown to elicit increases in blood pressure and heart rate in conscious normotensive rats (Nagahama et al., 1986a, 1987) and blood pressure in conscious spontaneously hypertensive rats (SHRs) after blockade of peripheral dopamine receptors (Van den Buuse, 1992; Van den Buuse et al., 1996). Decreases in heart rate and blood pressure were observed in pentobarbitone-anaesthetised normotensive rats (Nagahama et al., 1986b; Sengupta and Lokhandwala, 1985; Lefevre-Borg et al., 1987; Cavero et al., 1987), anaesthetised rhesus monkeys (Hahn and MacDonald, 1984) and in conscious SHRs (Hahn et al., 1983; Kurz et al., 1986; Lefevre-Borg et al., 1987). The latter effects are most likely mediated, at least in part, by inhibition of sympathetic noradrenergic neurotransmission through peripheral dopamine D_2 receptors (Langer, 1981; Cavero et al., 1982a; Willems et al., 1985). However, the pressor action of quinpirole in conscious normotensive rats

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is attributed to a central dopamine D_2 receptor-mediated activation of sympathetic outflow associated with arginine vasopressin release, whereas the tachycardiac response is dependent only on activation of sympathetic outflow, resulting in increased plasma catecholamine concentrations (Nagahama et al., 1986a, 1987). Thus, the central influence of quinpirole appears to predominate in conscious rats and to mask the depressor and bradycardic effects of this agonist at peripheral dopamine D_2 receptor sites. In view of the importance of the intermediolateral cell column in the thoracic spinal cord, as the origin of preganglionic sympathetic neurons, dopamine receptors in the spinal cord may also be a target for the cardiovascular effects induced by intravenous administration of quinpirole. The present study investigated this possibility. The diencephalo-spinal dopaminergic system, whose existence has now been well demonstrated by both biochemical and anatomical studies, has its origin in the dorsal medial hypothalamus–caudal thalamus group (A_{11} area in the rat) and projects mainly around the preganglionic sympathetic neurons in the thoracicolumbar spinal cord (Hökfelt et al., 1979; Skagerberg et al., 1982; for review, see Feuerstein et al., 1988). Spinal dopamine D_1 and D_2 receptors have been also characterized (Demenge et al., 1981; Dubois et al., 1986; Van Dijken et al., 1996) and are involved in nociceptive reactions (Jensen and Smith, 1982, 1983; Fleetwood-Walker et al., 1988; Weil-Fugazza and Godefroy, 1991) as well as in adrenal gland secretion (Arneric et al., 1984). Furthermore, it was shown that intrathecal administration at the upper thoracic level of apomorphine, a mixed dopamine D_1/D_2 receptor agonist, induced dose-dependent hypotension and bradycardia in conscious rats (Petitjean et al., 1984). Both the magnitude and the time of appearance of these effects varied according to the spinal level of administration and were elicited by stimulation of specific spinal dopamine receptors, located from the mid-cervical to low thoracic levels (Lahlou et al., 1990). The spinal origin of these effects was corroborated by the fact that spinal transection, performed at the midthoracic level (T5–T7), in order to interrupt the diencephalo-spinal dopaminergic system, enhanced the hypotension and bradycardia elicited by intrathecal apomorphine, when administered caudally (at T9–T10) but not rostrally (at T2–T4) to the spinal transection (Lahlou and Demenge, 1993). This spinal stimulation has been shown to be partly responsible for the hypotensive effects of intravenously administered bromocriptine, a dopamine D_2 receptor agonist (Lahlou and Demenge, 1991). The idea of this partial spinal mediation was corroborated by studies in spinal cord-transected rats (Lahlou and Demenge, 1992). In order to determine whether such a spinal mediation is characteristic of bromocriptine alone, the aim of the present study was to examine the possible participation of the diencephalo-spinal dopaminergic system in the cardiovascular effects of intravenously administered quinpirole in pentobarbitone-anaesthetised rats.

2. Materials and methods

2.1. Catheterisation procedure

Male Wistar rats, weighing 260–300 g, were kept under conditions of constant temperature (22–23°C) with a standard light/dark cycle (12-h light/12-h dark; lights on at 0700 h) and free access to food and water. They were anaesthetised with intraperitoneally injected sodium pentobarbitone (50 mg/kg) and three catheters were implanted and exteriorised at the dorsal neck level according to the following procedure. Two teflon catheters (0.40 mm internal diameter; Habia Cables, Montmirail, France), filled with heparinised (125 IU/ml) isotonic saline, were implanted in the abdominal aorta (for the recording of arterial pressure) and in the inferior vena cava (for drug administration), both up to 1 cm below the renal artery, as previously described (Petitjean et al., 1984). The third catheter (polyethylene tubing, PE 10), filled with isotonic saline, allowed low thoracic intrathecal administration of drugs. It was inserted into the spinal subarachnoid space through an incision made at T13, and its tip was advanced rostrally 1.5 to 1.8 cm to reach the T9–T10 spinal cord level. It was sealed in place with cyanoacrylate glue (Krazy Glue, Chicago, IL, USA) according to a previously described method (Takano et al., 1984). After surgery, the rats were housed individually in plastic cages and allowed to recover for 48 h. Only rats with no apparent motor deficit were used for the present studies.

At the end of the experiments, the rats were killed with intravenous pentobarbitone and their lower thoracic cords were examined by dye injection (bromophenol blue) to assess the correct positioning of the tip of the intrathecal catheter as well as the absence of catheter-induced spinal lesions. Individual results were discarded if these criteria were not met.

2.2. Recordings of mean aortic pressure and heart rate

During the experiments, the arterial catheter was connected to a blood pressure transducer coupled to a polygraph recorder; heart rate was obtained using a cardiotachometer triggered by the pressure pulses. Both signals were recorded on a Gilson model 5/6H polygraph (Gilson Medical Electronics, Middleton, WI, USA). Mean aortic pressure was calculated as diastolic + [(systolic − diastolic)/3].

2.3. Experimental protocol

On the day of experiments, the rats were again anaesthetised with sodium pentobarbitone (50 mg/kg) and rectal temperature was kept close to 37°C by placing the animals on a thermostatically controlled table. In order to explore the cardiovascular responses to quinpirole, the following protocol was used. Before each experiment,

performed at midday, blood pressure and heart rate were allowed to stabilize and both variables were recorded for 30 min after quinpirole or vehicle treatment. Rats with intrathecal catheters were challenged at the beginning of each experiment with an intrathecal injection of 10 μl of vehicle and only the rats exhibiting no significant changes were further used in this study (Petitjean et al., 1984; Lahlou et al., 1990). When the effects of an antagonist were tested, antagonist injection occurred 5 min before the administration of the agonist. When the effects of combined intrathecal and intravenous pretreatment with an antagonist were tested, the intravenous pretreatment commenced 10 min before agonist injection; however, intrathecal pretreatment began 5 min before agonist administration, as in all other experiments. Each set of experiments (i.e., complete dose–effect curve or challenge with an antagonist) was performed in five to nine animals per group. To avoid desensitization phenomena (Van den Buuse, 1992; Van den Buuse et al., 1996; Van den Buuse, 1997), only one dose of intrathecal or intravenous quinpirole could be administered per day. After experiments, the rats were returned to the animal room and sometimes used for another experiment at least 48 h later. This investigation conforms with the Guide for the Care and Use of Laboratory Animals, published by the US National Institute of Health (NIH Publication No 85-23, revised 1985). The following experiments were performed.

2.3.1. Series 1

This series of experiments was carried out to establish a dose–effect relationship: increasing bolus doses of quinpirole (25, 50, 150, 300 and 1000 μg/kg) were injected via the intravenous catheter and the time course of the changes in mean aortic pressure and heart rate was recorded. Control animals were injected intravenously with a volume of saline similar to that of quinpirole.

2.3.2. Series 2

Four different groups of animals were used in this series in order to elucidate the mechanisms of cardiovascular action of quinpirole. These experiments were performed with the dopamine D_2 receptor antagonist domperidone, which does not cross the blood-brain barrier (Laduron and Leysen, 1979; Sved and Fernstrom, 1980; Kohli et al., 1983; Champion, 1988). Therefore, the time course of the changes in mean aortic pressure and heart rate induced by intravenous quinpirole, at a dose (1000 μg/kg) previously used for the same purpose by Nagahama et al. (1986b), was determined in control rats (intravenous or intrathecal vehicle-treated; first group) and in rats which had been subjected to either peripheral blockade by intravenous domperidone (0.5 mg/kg; second group) (Nagahama et al., 1986b; Lahlou and Demenge, 1991), spinal blockade by intrathecal domperidone (40 μg/rat at T9–T10; third group) (Petitjean et al., 1984; Lahlou and Demenge, 1991) or combined peripheral and spinal blockade by intravenous (0.5 mg/kg, 10 min before) and intrathecal (40 μg/rat at T9–T10, 5 min before) domperidone (fourth group), respectively, of dopamine D_2 receptors.

2.3.3. Series 3

The time-course of the changes in mean aortic pressure and heart rate elicited by the same dose of quinpirole was also studied in an additional group of rats pretreated with an intravenous injection of 5 mg/kg (Nagahama et al., 1984, 1986b) metoclopramide, a dopamine D_2 receptor antagonist that crosses the blood–brain barrier (Peringer et al., 1976; Cavero et al., 1982b).

2.3.4. Series 4

Since hypotensive and bradycardic effects elicited by intravenous quinpirole were (see results of Section 2.3.2) reduced by intrathecally administered domperidone at the lower thoracic level (i.e., T9–T10), it was postulated that quinpirole applied intrathecally at the same level of spinal cord as above could decrease blood pressure and heart rate in a dose-related manner. This series of experiments was carried out to test this hypothesis. Therefore, increasing doses of quinpirole in the range of 7.7 to 61.4 μg/rat (30 to 240 nmol/rat) (Pellissier and Demenge, 1991) were injected intrathecally and the time course of the changes in mean aortic pressure and heart rate was recorded. Control animals were challenged intrathecally with an equal volume of saline.

2.3.5. Series 5

This series of experiments was undertaken to assess the origin and dopamine receptor specificity of the cardiovascular responses to intrathecal quinpirole. Therefore, the maximal changes in mean aortic pressure and heart rate induced by an intrathecal injection of quinpirole, at a dose (38.4 μg/rat) previously shown to elicit submaximal effects at the upper (Pellissier and Demenge, 1991) or the lower (the present study) thoracic level, were determined in control rats (intravenous or intrathecal vehicle-treated) and in rats which had been subjected to one of the following pretreatments: intrathecal haloperidol (10 μg/rat at T9–T10) (Petitjean et al., 1984; Lahlou and Demenge, 1993), intrathecal domperidone (40 μg/rat at T9–T10) or intravenous domperidone (0.5 mg/kg).

2.4. Drugs

Quinpirole hydrochloride (Research Biochemicals, Illkirsh, France) was dissolved in sterile isotonic saline solution immediately before use. Domperidone (Janssen Pharmaceuticals, Boulogne, France) was dissolved in tartaric acid (0.2%) and brought to the chosen volume with sterile isotonic saline just before use. Metoclopramide (Delagrange, Chilly-Mazarin, France), haloperidol (Janssen Pharmaceuticals) and sodium pentobarbitone (Sanofi, Libourne, France) were used as the commercially available

injectable solutions. For intravenous injection, drug or vehicle was given in a volume of 1 ml/kg of body weight followed by a 0.2-ml flush of physiological saline. For intrathecal administration, drug or vehicle was given in a volume of 10 μl and the catheter was flushed by using a Hamilton microsyringe with 10 μl (catheter volume) of saline to insure complete delivery of the dosage.

2.5. Statistical analysis

All the results are expressed as means ± S.E.M. Changes in absolute mean aortic pressure and heart rate with respect to individual baseline values were computed. A one-way analysis of variance (ANOVA = groups) and unpaired Student's *t*-tests were selected to assess significant differences in baseline mean aortic pressure and heart rate between the different groups. The paired Student's *t*-test was used to investigate significant changes in cardiovascular variables following intrathecal or intravenous administration of vehicle or dopamine receptor antagonists. The statistical significance of mean aortic pressure and heart rate responses to quinpirole alone or in association with a dopamine receptor antagonist compared with those of saline-treated animals was determined by Mann–Whitney *U*-tests. Groups of data were compared with one-way (doses) or two-way (treatment × time) analysis of variance (ANOVA), followed by Tukey's significant difference test where appropriate. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Cardiovascular effects of intravenous quinpirole at increasing bolus doses (Series 1)

Basal mean aortic pressure and heart rate were 103.2 ± 1.4 mmHg and 358 ± 6 beats/min (six groups of 5–8 rats each), respectively, and there were no significant differences between groups ($P > 0.05$, one-way ANOVA). In control animals, neither blood pressure nor heart rate changed significantly during the 30-min period after vehicle administration (Fig. 1). In contrast, intravenous injection of quinpirole, in a 25 to 1000 μg/kg dosage range, decreased mean aortic pressure and heart rate in a dose-related fashion (Fig. 1). The quinpirole-induced hypotension became significant at the dose of 25 μg/kg (Fig. 1; $P < 0.01$, Mann–Whitney *U*-test) while the bradycardic response was significant only for the dosage of 50 μg/kg (Fig. 1; $P < 0.05$, Mann–Whitney *U*-test). Hypotensive and bradycardic effects peaked within the first 1 min and 3–5 min after quinpirole treatment, respectively (Fig. 1). For both effects, time to maximal effect was similar for each dose, but the duration was dose related. At the lowest doses (25 and 50 μg/kg) studied, there was a partial recovery towards pre-dose values within 5–15 min following quinpirole treatment, but mean aortic pressure and heart rate remained significantly reduced 30 min after administration of higher dosages (Fig. 1; $P < 0.01$, Mann–Whitney *U*-test). Furthermore, maximal mean aortic pressure and heart rate responses were significantly correlated to the dose of quinpirole (Fig. 1; $P < 0.001$, one-way ANOVA).

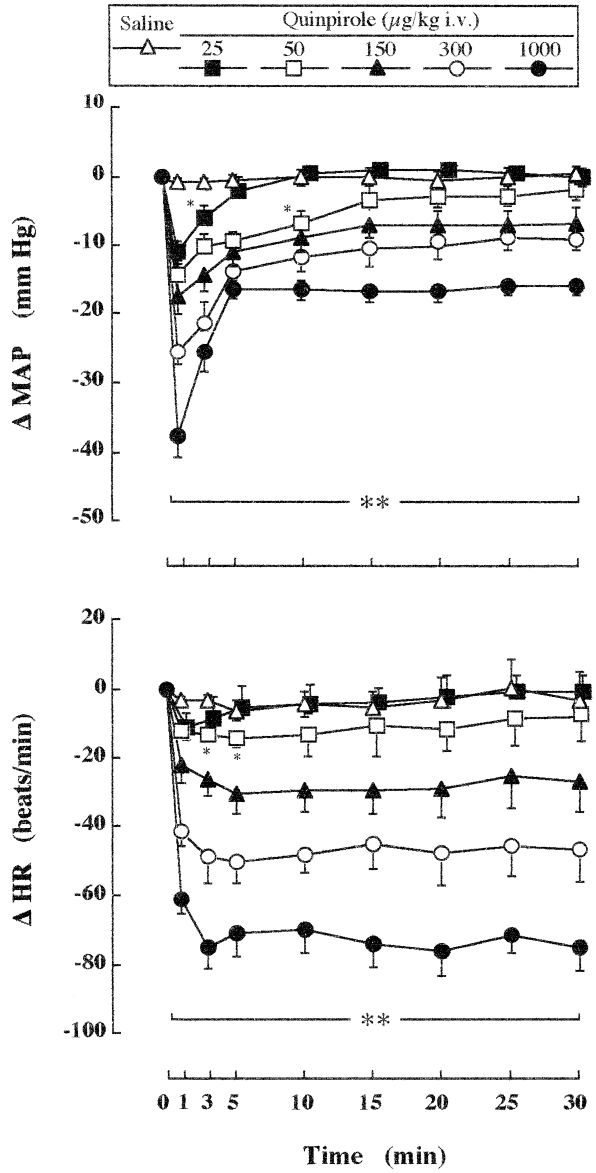

Fig. 1. Time-course of the changes in mean aortic pressure (MAP) (ΔMAP; upper panel) and heart rate (HR) (ΔHR; lower panel) elicited by bolus doses (25 to 1000 μg/kg) of intravenous quinpirole in pentobarbitone-anaesthetised rats. Vertical bars indicate S.E.M. (5–8 rats per group). Baseline MAP and HR values were 103.2 ± 1.4 mmHg and 358 ± 6 beats/min, respectively (six groups). Intravenous quinpirole elicited significant decreases in MAP and HR. For both effects, time to maximal effect was similar for each dose, whereas the duration was dose related. The maximal changes in MAP and HR were significantly related to the dose of quinpirole ($P < 0.001$, one-way ANOVA). * $P < 0.05$, ** $P < 0.01$ by Mann–Whitney *U*-test with respect to animals challenged with intravenous saline.

3.2. Effects of intravenous and / or intrathecal pretreatment with domperidone on cardiovascular actions of intravenous quinpirole (Series 2)

In this series of experiments, mean baseline aortic pressure and heart rate were 103.0 ± 1.3 mmHg and 356 ± 6 beats/min (six groups of 6–9 rats), respectively and remained very stable during the 30 min that followed

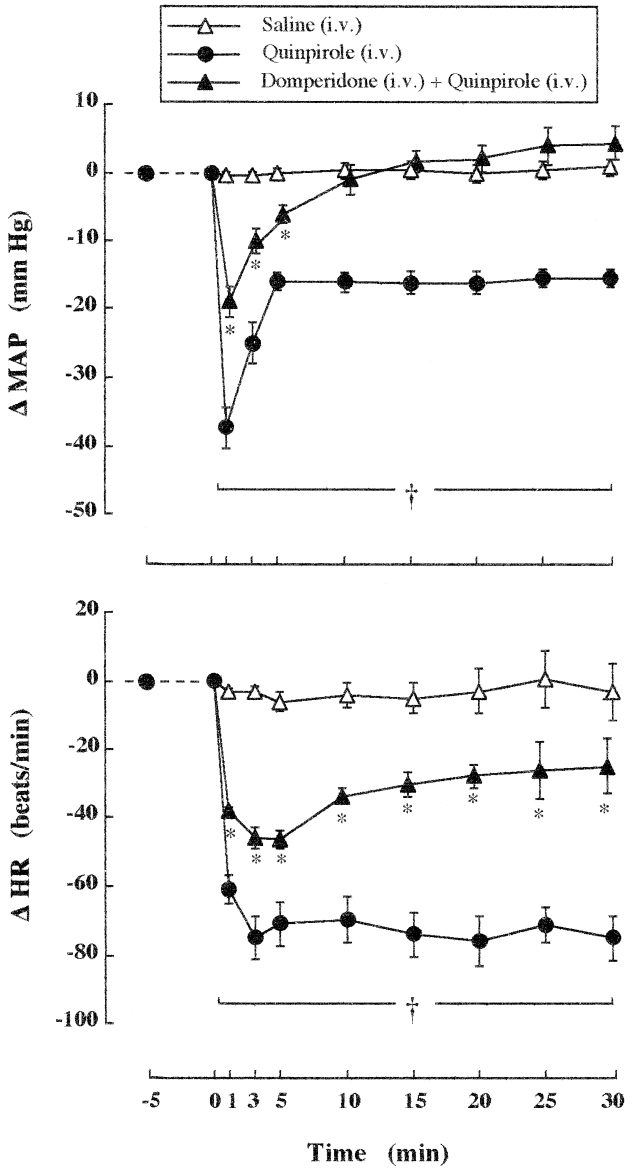

Fig. 2. Time-course of the changes in MAP (ΔMAP; upper panel) and HR (ΔHR; lower panel) elicited by intravenous treatment with quinpirole (1000 μg/kg) without or with intravenous domperidone (0.5 mg/kg) pretreatment in pentobarbitone-anaesthetised rats. Vertical bars indicate S.E.M. (6–9 rats per group). Baseline MAP and HR values were 103.0 ± 1.4 mmHg and 356 ± 6 beats/min, respectively (three groups). Maximal hypotensive and bradycardic responses to quinpirole were significantly, but not completely, blocked by pretreatment with intravenous domperidone ($^{\dagger}P < 0.001$, two-way ANOVA followed by the Tukey's significant difference test). The remaining decreases in MAP and HR were significant ($* P < 0.01$, Mann–Whitney *U*-test) with respect to animals challenged with intravenous saline.

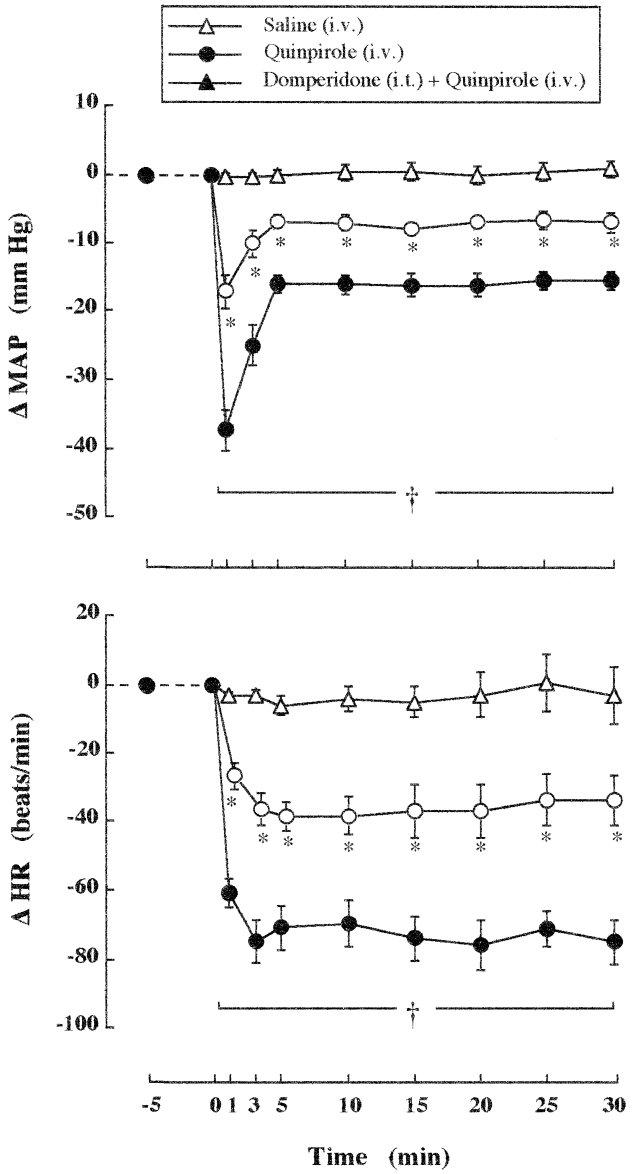

Fig. 3. Time-course of the decreases in MAP (ΔMAP; upper panel) and HR (ΔHR; lower panel) elicited by intravenous treatment with quinpirole (1000 μg/kg) without or with intrathecal domperidone (40 μg/rat at T9–T10) pretreatment in pentobarbitone-anaesthetised rats. Vertical bars indicate S.E.M. (6–9 rats per group). Baseline MAP and HR values were 103.3 ± 1.5 mmHg and 353 ± 7 beats/min, respectively (three groups). Intrathecal pretreatment with domperidone decreased partially, but significantly, the magnitude of hypotensive and bradycardic responses to intravenously administered quinpirole ($^{\dagger}P < 0.001$; two-way ANOVA). The remaining decreases in MAP and HR were significant ($* P < 0.01$; Mann–Whitney *U*-test) with respect to those of animals challenged with intravenous saline.

vehicle administration in control experiments. Furthermore, there were no significant differences in baseline values between groups ($P > 0.05$, one-way ANOVA). After pretreatment with vehicle, intravenous quinpirole (1000 μg/kg) produced significant ($P < 0.001$, Mann–Whitney *U*-test) decreases in mean aortic pressure and heart rate, the magnitude of which was maximal (−37 ± 3 mmHg

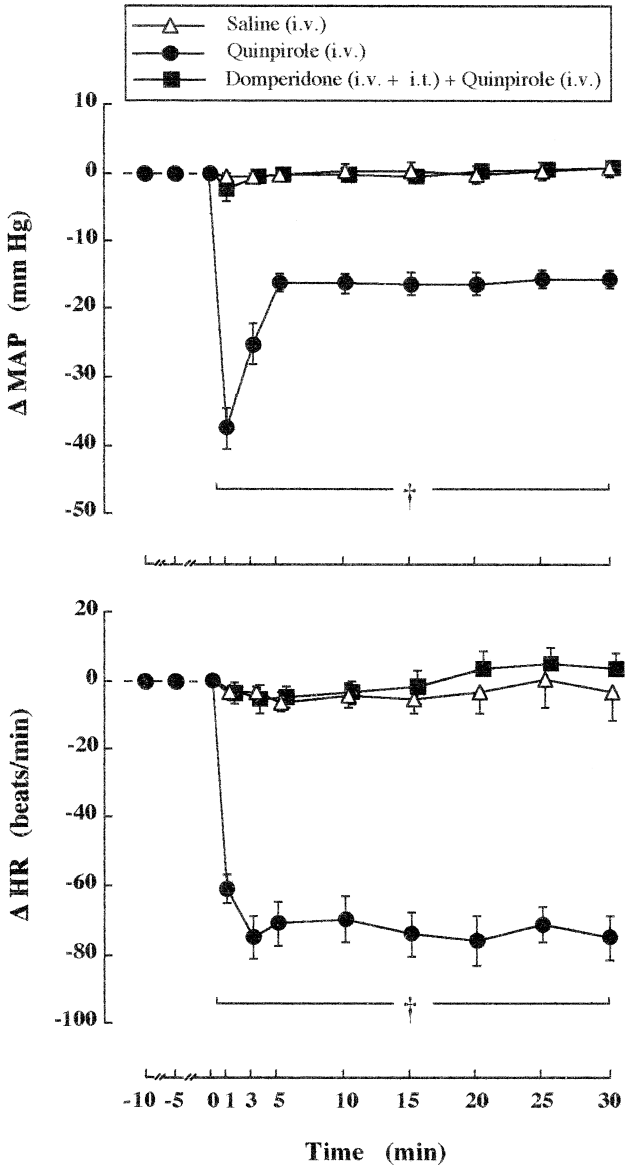

Fig. 4. Time-course of the changes in MAP (ΔMAP; upper panel) and HR (ΔHR; lower panel) elicited by intravenous treatment with quinpirole (1000 μg/kg) without or with both intravenous (0.5 mg/kg) and intrathecal (40 μg/rat at T9–T10) domperidone pretreatment in pentobarbitone-anaesthetised rats. Vertical bars indicate S.E.M. (6–9 rats per group). Baseline MAP and HR values were 102.7 ± 1.9 mmHg and 352 ± 7 beats/min, respectively (three groups). Pretreatment with both intravenous and intrathecal domperidone fully abolished the hypotensive and bradycardic effects elicited by intravenous quinpirole ($^{\dagger}P < 0.001$, two-way ANOVA).

and -75 ± 6 beats/min, respectively) within the first 1 and 3 min following drug treatment, respectively (Figs. 2–4).

3.2.1. Effects of intravenous pretreatment with domperidone on the cardiovascular actions of intravenous quinpirole

Pretreatment with intravenous domperidone (0.5 mg/kg) did not alter baseline mean aortic pressure (103.2 ± 2.2 vs. 102.6 ± 1.5 mmHg) or heart rate (352 ± 12 vs. 351 ± 13 beats/min) ($P > 0.05$, paired Student's *t*-test); however, it significantly reduced the maximal hypotensive and bradycardic responses elicited by intravenous quinpirole (1000 μg/kg) by nearly 48% and 42%, respectively (Fig. 2; $P < 0.001$, two-way ANOVA). The remaining decrease in mean aortic pressure (52% 1 min after administration) was still statistically significant with respect to control values for 5 min (Fig. 2; $P < 0.01$, Mann–Whitney *U*-test) and was followed by a small, but non-significant pressor response ($P > 0.05$, Mann–Whitney *U*-test). In contrast, the residual bradycardic response (nearly 58% 3 min after administration) remained statistically significant with respect to control values during the whole experimental period (Fig. 2; $P < 0.01$, Mann–Whitney *U*-test).

3.2.2. Effects of intrathecal pretreatment with domperidone on the cardiovascular actions of intravenous quinpirole

In rats, which had been pretreated with intrathecal domperidone (40 μg/rat at T9–T10), the maximal hypotensive and bradycardic responses to intravenous quinpirole (1000 μg/kg) were also significantly reduced by nearly 54% and 49%, respectively (Fig. 3; $P < 0.001$, two-way ANOVA). Residual decreases in mean aortic pressure and heart rate after intrathecal domperidone peaked within the first 1 min and 5 min after quinpirole treatment, respectively, and remained significant with respect to those of animals challenged with vehicle during the 30-min observation period (Fig. 3; $P < 0.01$, Mann–Whitney *U*-test). Intrathecal domperidone did not affect ($P > 0.05$, paired Student's *t*-test) baseline mean aortic pressure (102.0 ± 2.1 vs. 101.0 ± 1.5 mmHg) or heart rate (356 ± 12 vs. 349 ± 7 beats/min).

3.2.3. Effects of combined intravenous and intrathecal pretreatment with domperidone on the cardiovascular actions of intravenous quinpirole

When quinpirole (1000 μg/kg) was injected intravenously in animals pretreated with both intravenous and intrathecal domperidone, its hypotensive and bradycardic effects were significantly blocked (Fig. 4; $P < 0.001$, two-way ANOVA). A two-way ANOVA revealed that quinpirole-induced changes in mean aortic pressure and heart rate after combined intravenous and intrathecal domperidone pretreatment were not statistically ($P > 0.05$) different from those obtained with vehicle injection alone. As mentioned earlier, intravenous or intrathecal domperidone was without significant effect on either basal mean aortic pressure or heart rate.

3.3. Effects of intravenous pretreatment with metoclopramide on the cardiovascular actions of intravenous quinpirole (Series 3)

The intravenous pretreatment with metoclopramide (5 mg/kg) did not modify ($P > 0.05$, paired Student's *t*-test)

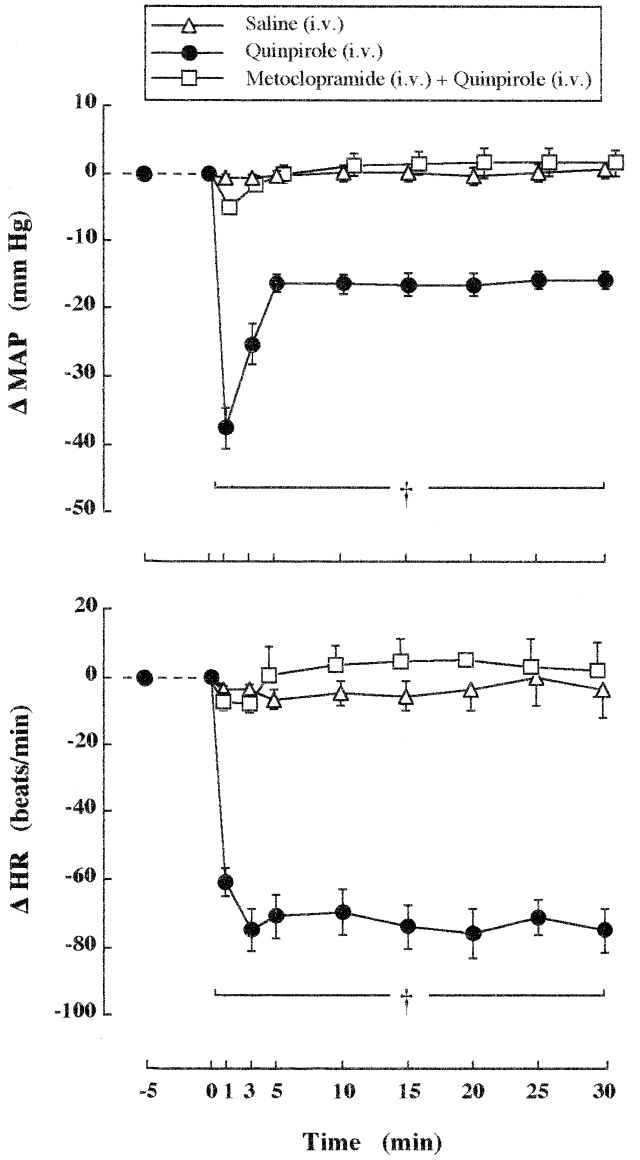

Fig. 5. Time-course of the changes in MAP (ΔMAP; upper panel) and HR (ΔHR; lower panel) elicited by intravenous treatment with quinpirole (1000 μg/kg) without or with intravenous metoclopramide (5 mg/kg) pretreatment in pentobarbitone-anaesthetised rats. Vertical bars indicate S.E.M. (6–9 rats per group). Baseline MAP and HR values were 102.2 ± 1.4 mmHg and 354 ± 7 beats/min, respectively (three groups). Quinpirole-induced decreases in MAP and HR were significantly suppressed by pretreatment with intravenous metoclopramide ($^{\dagger}P < 0.001$, two-way ANOVA).

baseline mean aortic pressure (102.0 ± 2.0 vs. 101.7 ± 2.1 mmHg) or heart rate (357 ± 12 vs. 351 ± 11 beats/min) but significantly suppressed the hypotension and bradycardia after intravenous quinpirole (Fig. 5; $P < 0.001$, two-way ANOVA). The changes in mean aortic pressure and heart rate elicited by quinpirole in metoclopramide-pretreated rats were not statistically ($P > 0.05$, two-way ANOVA) different from those obtained in animals pretreated with both intravenous and intrathecal domperidone.

3.4. Cardiovascular effects of intrathecal quinpirole at increasing doses (Series 4)

In this series of experiments, mean baseline aortic pressure and heart rate were 103.4 ± 1.4 mmHg and 362 ± 9 beats/min (six groups of 5–8 rats), respectively, and were not significantly different between groups ($P > 0.05$, one-way ANOVA). In control animals, both variables re-

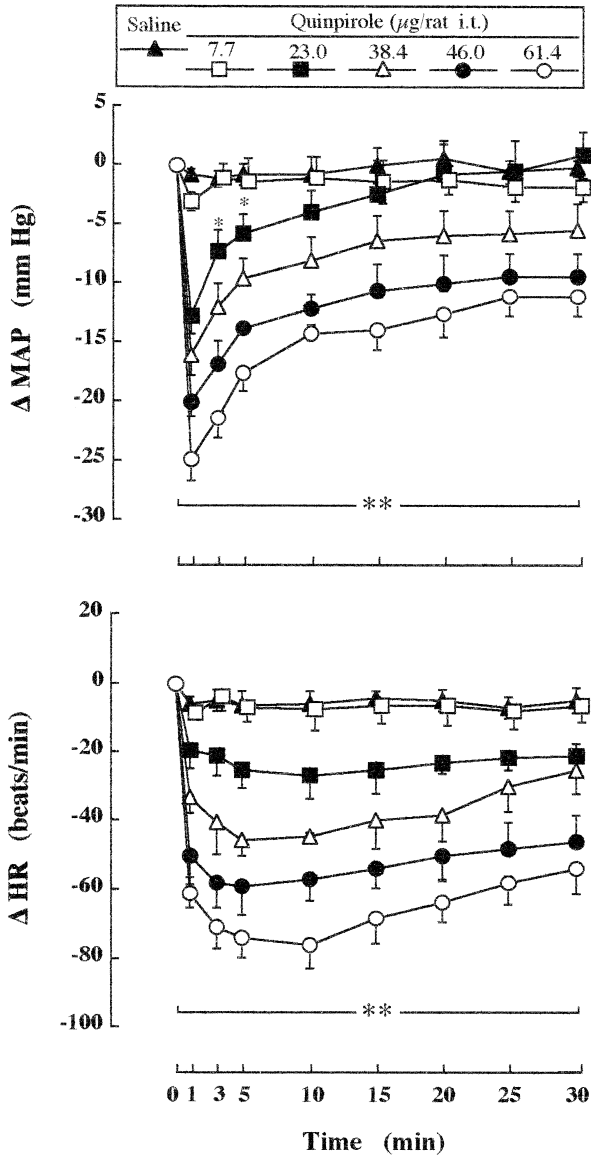

Fig. 6. Time-course of the changes in MAP (ΔMAP; upper panel) and HR (ΔHR; lower panel) elicited by increasing doses (7.7–61.4 μg/rat) of intrathecal quinpirole injected at the T9–T10 level in pentobarbitone-anaesthetised rats. Vertical bars indicate S.E.M. (5–8 rats per group). Baseline MAP and HR values were 103.4 ± 1.4 mmHg and 362 ± 9 beats/min, respectively (six groups). Intrathecal treatment with quinpirole produced significant hypotension and bradycardia. For both effects, time to maximal effect was similar for each dose, whereas the duration was dose related. Maximal changes in MAP and HR were significantly related to the dose of quinpirole ($P < 0.001$, one-way ANOVA). $*P < 0.05$, $**P < 0.01$ by Mann–Whitney *U*-test with respect to animals challenged with intrathecal saline.

mained stable during the 30 min following intrathecal treatment with 10 μl of saline (Fig. 6; $P > 0.05$, Student's *t*-test). In contrast, intrathecal injection of increasing doses (7.7 to 61.4 μg/rat) of quinpirole at the T9–T10 level significantly decreased mean aortic pressure and heart rate (Fig. 6). Both effects became significant at the dose of 23 μg/rat (Fig. 6; $P < 0.01$, Mann–Whitney *U*-test). For all doses of intrathecal quinpirole, hypotension appeared immediately and bradycardia was slightly delayed (30 s post-injection). These responses were maximal within the first 1 min and 5–10 min following quinpirole treatment, respectively, after which blood pressure and heart rate gradually returned to mean basal levels (Fig. 6). Maximal decreases in mean aortic pressure and heart rate were significantly correlated to the dose of quinpirole (Fig. 6; $P < 0.001$, one-way ANOVA).

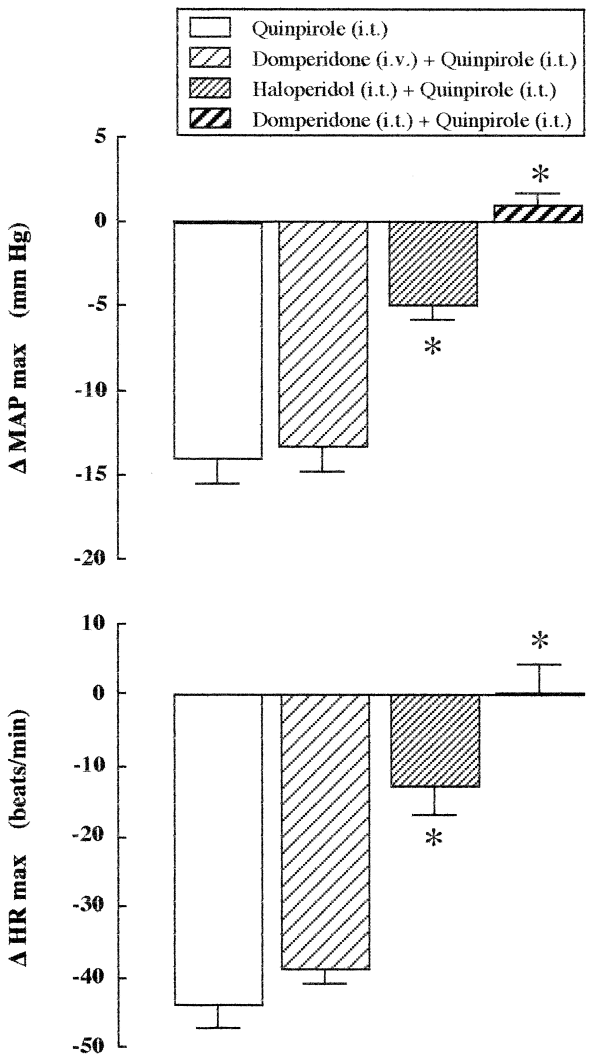

Fig. 7. Effects of intrathecal pretreatment (at T9–T10) with either haloperidol (10 μg/rat) or domperidone (40 μg/rat) and of intravenous pretreatment with domperidone (0.5 mg/kg) on the maximal changes in MAP (ΔMAP max; upper panel) and HR (ΔHR max; lower panel) elicited by intrathecal administration of quinpirole (38.4 μg/rat at T9–T10) in pentobarbitone-anaesthetised rats. Vertical bars indicate S.E.M. (6–9 rats per group). Baseline MAP and HR values were 104.0 ± 1.2 mmHg and 368 ± 6 beats/min, respectively (four groups). Maximal quinpirole-induced hypotensive and bradycardic responses remained unchanged by intravenous domperidone pretreatment while they were significantly reduced and blocked respectively by haloperidol and domperidone given intrathecally. $* P < 0.001$ by Mann–Whitney *U*-test with respect to animals challenged with intrathecal quinpirole.

3.5. Origin and dopamine receptor specificity of the cardiovascular effects of intrathecal quinpirole (Series 5)

In vehicle-pretreated rats, intrathecal injection of the submaximal dose (38.4 μg/rat at T9–T10) of quinpirole induced significant decreases in mean aortic pressure and heart rate ($P < 0.001$, Mann–Whitney *U*-test) which were maximal within the first 1 min and 5 min after drug treatment, respectively. Maximal hypotensive (-14.0 ± 1.5 mmHg) and bradycardic (-44 ± 4 beats/min) responses to intrathecal quinpirole were significantly (Fig. 7; $P < 0.001$, Mann–Whitney *U*-test) reduced and fully suppressed by intrathecal (at T9–T10) pretreatment with haloperidol (10 μg/rat) and domperidone (40 μg/rat), respectively, while they remained unchanged (Fig. 7; $P > 0.05$, Mann–Whitney *U*-test) after intravenous pretreatment with domperidone (0.5 mg/kg). Intrathecal haloperidol did not induce significant ($P > 0.05$, paired Student's *t*-test) changes in either mean aortic pressure (103.6 ± 2.0 vs. 104.0 ± 2.2 mmHg) or heart rate (359 ± 12 vs. 363 ± 11 beats/min). As in Series 2, baseline values of both variables were unaffected by either intravenous or intrathecal domperidone.

4. Discussion

The baseline mean aortic pressure and heart rate of pentobarbitone-anaesthetised rats with intrathecally implanted catheters were similar to those reported by others laboratories (Kubo et al., 1987; Martinez-Arizala et al., 1989; Gradin, 1990; Pellissier and Demenge, 1991) using the same preparation.

The present study reports that intravenously administered quinpirole dose dependently decreased mean aortic pressure and heart rate. This agrees with previous studies regarding the cardiovascular effects of quinpirole in pentobarbitone-anaesthetised normotensive rats (Sengupta and Lokhandwala, 1985; Nagahama et al., 1986b; Lefevre-Borg et al., 1987; Cavero et al., 1987). However, the magnitude of the depressor effect of quinpirole at 1 mg/kg was smaller than that found with the same dose in an earlier report (Nagahama et al., 1986b), although Nagahama et al. did not investigate the dose–effect relationship. Such a discrepancy may result from differences either in mean arterial pressure at baseline (103.2 ± 1.4 vs. 115.7 ± 1.2 mmHg in the present and the Nagahama study, respectively) and/or in the rat strain used (Wistar vs. Sprague–Dawley, respectively). Conversely, the time course of the

decreases in mean aortic pressure and heart rate evoked by quinpirole was similar for both studies. Although quinpirole has weak α_2-adrenoceptor and histamine H_2 agonist properties (Cohen et al., 1984; Ruffolo and Shaar, 1983; Armstrong et al., 1983), its hypotensive and bradycardic effects can be attributed mostly to stimulation of dopamine D_2 receptors, as they are suppressed by pretreatment with metoclopramide, a dopamine D_2 receptor antagonist that crosses the blood-brain barrier. Some of these receptors are located outside the central nervous system since pretreatment with intravenous domperidone, which does not cross the blood-brain barrier, partially reduced the maximal quinpirole-induced hypotension and bradycardia. This conclusion is supported by previous results obtained in anaesthetised normotensive rats (Nagahama et al., 1986b; Sengupta and Lokhandwala, 1985; Lefevre-Borg et al., 1987; Cavero et al., 1987).

However, the current study showed that once spinal dopamine D_2 receptors are blocked with intrathecal domperidone, the decreases in mean aortic pressure and heart rate elicited by intravenous administration of quinpirole are also partly, but significantly, reduced, indicating that they are at least partly mediated by spinal dopamine D_2 receptor stimulation. To the best of our knowledge, this is the first time that partial spinal mediation of the cardiovascular effects of intravenous quinpirole in anaesthetised rats has been demonstrated. We have previously reported that intravenous bromocriptine, another dopamine D_2 receptor agonist, can partly decrease blood pressure by activation of spinal dopamine D_2 receptors in both anaesthetised and conscious normotensive rats (Lahlou and Demenge, 1991). Thus, the current results replicate our earlier findings and suggest that the partial spinal mediation may not be peculiar to bromocriptine, but may be common to most active dopamine D_2 receptors agonists. Hypotensive and bradycardic responses to intravenous quinpirole appear to be fully mediated by dopamine D_2 receptors, some of which are located within the spinal cord and some of which are located in the peripheral circulation, since both responses were completely abolished by combined intrathecal and intravenous domperidone pretreatment.

Pretreatment with intravenous domperidone completely blocked the quinpirole-induced hypotension at 10 min post-injection and resulted in a pressor response that was statistically non-significant. In view of this observation, it seemed possible that quinpirole might have a central pressor effect which could be masked by the residual spinal depressor component. Thus, one might have expected that without the spinal depressor component, the pressor effect of quinpirole would have appeared more rapidly and might have become statistically significant. However, this possibility appears unlikely because blockade of both peripheral and spinal dopamine D_2 receptors with domperidone did not reveal a pressor effect of quinpirole, at least under the experimental conditions used. Moreover, the quinpirole-induced changes in mean aortic pressure and heart rate after intravenous pretreatment with metoclopramide were not statistically different from those observed after combined pretreatment with intravenous and intrathecal domperidone. It is important to note, however, that in pentobarbitone-anaesthetised Sprague–Dawley rats, although pretreatment with a vasopressin antagonist was unable to enhance the maximal quinpirole-induced depressor response, it significantly delayed the recovery phase of quinpirole-induced hypotension (Nagahama et al., 1986b). Thus, the role of a quinpirole-induced increase in plasma levels of vasopressin in the cardiovascular action of this agonist should be considered, at least in anaesthetised Sprague–Dawley rats.

The hypothesis of the involvement of spinal dopamine D_2 receptors in mediation of the cardiovascular responses to intravenous quinpirole is strongly supported by two lines of evidence. First, the hypotension and bradycardia remaining after intravenous domperidone pretreatment were of the same order of magnitude as those elicited by intrathecally administered domperidone. In fact, because intrathecal domperidone blocked nearly 54% and 49% of the maximal quinpirole-induced hypotension and bradycardia, respectively, intravenous domperidone could not have inhibited these variables by more than 46% and 51%, respectively, and it did not. Moreover, if the decreases in mean aortic pressure and heart rate induced by quinpirole after intravenous domperidone are not of spinal dopaminergic origin, then they would not have been affected by the additional pretreatment with intrathecal domperidone. However, the elimination of the remaining quinpirole-induced responses by intrathecal domperidone pretreatment makes it clear that spinal dopamine D_2 receptors are involved.

Second, intrathecal injection of quinpirole at the T9–T10 level was able to induce significant and dose-dependent decreases in mean aortic pressure and heart rate through a spinal dopaminergic mechanism. It is interesting to note that intrathecal quinpirole-induced hypotension and bradycardia peaked within the first 1 min and 5–10 min after injection, respectively, a time course which is identical to that of the residual hypotension and bradycardia induced by intravenous quinpirole after intravenous domperidone pretreatment (i.e., the spinal component of the responses). Hypotensive and bradycardic effects induced by intrathecal quinpirole are related to dopamine receptor activation since they were significantly reduced by intrathecal haloperidol, a non-selective dopamine receptor antagonist. The involvement of dopamine D_2 receptors is strengthened by the finding that quinpirole-induced decreases in blood pressure and heart rate were blocked by intrathecal domperidone. These results are in agreement with those of Pellissier and Demenge (1991), who injected quinpirole at the upper thoracic (i.e., T2–T4) level of the spinal cord. Convincing experimental arguments allowed the assignment of a spinal site of action for the cardiovascular responses to intrathecal quinpirole and excluded the possibility that this agonist

may have diffused from the subarachnoid space to brain stem structures or into the circulation to produce the cardiovascular effects reported here, through a central or peripheral dopaminergic mechanism, respectively. The rapid onset (few seconds) of responses to intrathecal quinpirole implies such a spinal site of action, which is probably confined to a restricted area close to the site of administration, as demonstrated in our previous study with tritiated apomorphine (Lahlou et al., 1990). Central mediation seems unlikely since quinpirole given via lateral cerebral ventricle injection in pentobarbitone-anaesthetised rats decreased mean arterial pressure through a peripheral rather than central dopaminergic mechanism (Lefevre-Borg et al., 1987). Peripheral mediation also seems unlikely since intrathecal, but not intravenous, pretreatment with domperidone counteracted the cardiovascular effects elicited by intrathecal injection of quinpirole. Thus, the present data point to the possible participation of the diencephalo-spinal dopaminergic system in the cardiovascular effects of intravenously administered quinpirole in pentobarbitone-anaesthetised rats.

The fact that the blockade of spinal dopamine receptors by intrathecally injected domperidone or haloperidol failed to alter the cardiovascular variables in anaesthetised rats indicates that spinal dopaminergic pathways controlling blood pressure and heart rate are not activated under normal conditions, at least in Wistar rats. This agrees with previous observations in conscious, freely moving rats (Petitjean et al., 1984; Lahlou et al., 1990; Lahlou and Demenge, 1993). Finally, the part of the hypotension and bradycardia attributed to spinal dopamine D_2 receptor activation may be masked by the central influence of intravenous quinpirole in conscious rats, as was suggested for the peripheral sympathoinhibitory depressor effect (Nagahama et al., 1986b). Further investigations are presently underway in our laboratory to test the latter hypothesis.

In conclusion, this study demonstrated that intravenous administration of quinpirole to pentobarbitone-anaesthetised rats decreases mean aortic pressure and heart rate partly through a peripheral dopaminergic mechanism and partly through stimulation of spinal dopamine D_2 receptors, as previously demonstrated for bromocriptine-induced hypotension. Hence, this spinal participation, which can be estimated at nearly 50% of the maximal quinpirole-induced hypotension and bradycardia, may not be peculiar to bromocriptine, as was previously thought. Rather, partial spinal mediation may be common to most active dopamine D_2 receptor agonists.

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