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Short communication

Mivazerol, a selective α_2-adrenoceptor agonist, attenuates tachycardia by intrathecal injection of *N*-methyl-D-aspartate in the rat

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Abstract

The intravenous (i.v.) infusion of mivazerol, a new selective α_2-adrenoceptor agonist, produced a significant decrease in heart rate but not in blood pressure in pentobarbital-anesthetized Sprague-Dawley rats. The tachycardic response to intrathecal (i.t.) injection of *N*-methyl-D-aspartic acid (NMDA) was significantly attenuated by the i.v. infusion of mivazerol. The i.t. pretreatment with yohimbine significantly attenuated the bradycardic response to i.v. mivazerol and blocked the effect of mivazerol on the tachycardic response to i.t. NMDA. These results suggest that (1) the bradycardic effect of mivazerol is mediated, at least partly, by spinal α_2-adrenoceptors; and (2) there is a possibility of functional antagonism between spinal α_2-adrenoceptors and NMDA receptors in the regulation of heart rate.

Keywords: Mivazerol; α_2-Adrenoceptor agonist; Heart rate; NMDA receptor; Spinal cord

1. Introduction

Since myocardial ischemia and adverse cardiac complications during the perioperative period are usually preceded by, and associated with hemodynamic changes (Goldman, 1995; Mangano et al., 1990) pharmacological control of hemodynamic instability may decrease the incidence of these postoperative complications. Several lines of evidence suggest that α_2-adrenoceptor agonists might be effective in blunting the perioperative stress response (Flacke et al., 1987; Ghignone et al., 1987) and that clonidine may have perioperative anti-ischemic effects (Dorman et al., 1993). Classical α_2-adrenoceptor agonists such as clonidine and dexmedetomidine, however, produce hypotension (Kallio et al., 1989; Kobinger, 1978), which is one of the factors contributing to intraoperative myocardial infarction (Cunningham, 1993). Mivazerol (3-[1(*H*-imidazol-4-yl)methyl]-2-hydroxybenzamide hydrochloride) is a new selective and specific α_2-adrenoceptor agonist (Noyer et al., 1994) which, contrary to classical specific α_2-adrenoceptor agonists, does not produce hypotensive effects (Roekaerts et al., 1996; Zhang et al., 1996).

It has been demonstrated that mivazerol prevents the immediate surges in heart rate and blood pressure observed in rats during emergence from halothane anesthesia (Guyaux et al., 1995). Recently, clinical phase II studies have also demonstrated that constant infusion of mivazerol prevents tachycardia and reduces the incidence of myocardial ischemia during the entire perioperative period in vascular surgery patients with, or at risk for coronary artery insufficiency (The McSPI Group, in press).

Many neurotransmitter systems are involved in the spinal regulation of heart rate. Considerable evidence supports the view that spinal α_2-adrenoceptor-mediated mechanisms are involved in the central control of heart rate (Gradin et al., 1992; Kubo et al., 1987). Moreover, there is much evidence that the glutamatergic system in the spinal cord exerts a stimulatory influence on the cardiovascular system and that NMDA receptors are involved in this modulation (Bazil and Gordon, 1993; West and Huang, 1994). However, the functional interaction between spinal α_2-adrenoceptors and NMDA receptors in the central control of heart rate remains unclear.

Therefore, the present study was performed to investigate the mechanism of the bradycardic action of mivazerol,

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focussing on the possibility of a functional interaction between spinal α_2-adrenoceptors and NMDA receptors in the control of heart rate.

2. Materials and methods

Male Sprague-Dawley rats weighing 250–350 g were anesthetized with pentobarbital sodium (60 mg/kg, i.p.) and placed in a stereotaxic instrument. Using a method described by Yaksh and Rudy (1976), a polyethylene tube (PE-10) was advanced about 3.2 cm from the lowest margin of the occipital bone to the second thoracic (T_2) vertebral level which corresponds to the principal level of sympathetic preganglionic neurons regulating cardiac functions (Jansen et al., 1995). A knot of the PE-10 tubing itself, which was made in the portion about 3.5 cm from the caudal tip of the PE-10 tubing, was fixed between the muscles. All layers of the wound were sutured. The i.t. catheter was flushed with 10 μl saline and the externalized rostral end of the catheter was closed by heating. Any subjects that exhibited motor deficits during 5 days between implantation of the i.t. catheter and testing were not used.

Rats which had been catheterized i.t. 5 days earlier, were anesthetized with pentobarbital sodium: initially with 60 mg/kg, i.p. and from about 30 min later until the end of experiment, with continuous i.v. infusion at the rate of 22 mg/kg per h. After trachea cannulation, rats were maintained under assisted air respiration using a ventilator (Model 683, Harvard). Blood pressure and heart rate were continuously monitored via a femoral arterial catheter (PE 50) connected to a transducer of a blood pressure analyzer (Model 100, Micro-Med). The femoral venous catheter (PE 50) was connected to a syringe mounted on a syringe pump (Model 341B, Sage Instruments) for i.v. infusion. Rectal temperature was maintained at 37.5 ± 0.5°C with a body temperature controller (Model CMA150, Carnegie Medicin). Thirty minutes after the start of i.v. infusion of mivazerol or vehicle at a rate of 0.1 ml/min, i.t. injection was made at a volume of 5 μl compound solution plus 5 μl saline flush with a Hamilton syringe, over an interval of 1 min. All compound solutions except yohimbine, which needs to be dissolved in distilled water, were prepared in sterile saline (0.9% NaCl). At the end of experiments, the quality of i.t. catheterization was examined after laminectomy.

Data are expressed as means ± S.E.M. Student's *t*-test was used for unpaired data. Differences between various means were determined by analysis of variance (ANOVA) followed by Scheffe's test. Statistical significance was accepted for $P < 0.05$.

3. Results

Mean arterial pressure and heart rate were maintained at a stable level during the experimental period by the i.v. supplemental infusion of pentobarbital sodium (22 mg/kg

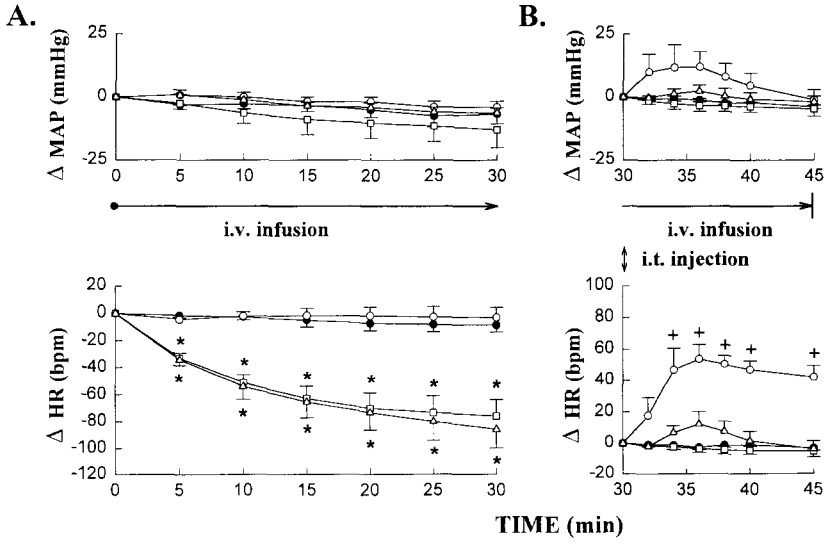

Fig. 1. A: Changes in mean arterial pressure (MAP) and heart rate (HR) during i.v. infusion of mivazerol or vehicle in the rats. B: Changes in mean arterial pressure and heart rate after i.t. injection of *N*-methyl-D-aspartic acid (NMDA) or saline in the vehicle- or mivazerol-i.v. infused rats. NMDA (15 nmol/kg) or saline (10 μl) were postinjected i.t. at the T_2 level 30 min after the start (0 min) of i.v. infusion of mivazerol (15.3 μg/kg per h) or vehicle (0.1 ml/min). Data are expressed as means ± S.E.M. Baseline values of mean arterial pressure (mmHg) and heart rate (bpm) at 0 min: 116.4 ± 7.7 and 439.6 ± 19.2 in the group of i.v. vehicle plus i.t. saline ($n = 5$, closed circles); 127.4 ± 9.0 and 427.6 ± 25.8 in the group of i.v. vehicle plus i.t. NMDA ($n = 5$, open circles); 124.0 ± 10.5 and 415.6 ± 25.7 in the group of i.v. mivazerol plus i.t. saline ($n = 5$, open squares); 122.0 ± 3.9 and 419.2 ± 17.6 in the group of i.v. mivazerol plus i.t. NMDA ($n = 5$, open triangles). * $P < 0.01$ vs. vehicle-i.v. infused groups; + $P < 0.01$ vs. group of i.v. mivazerol plus i.t. NMDA, and group of i.v. vehicle plus i.t. saline.

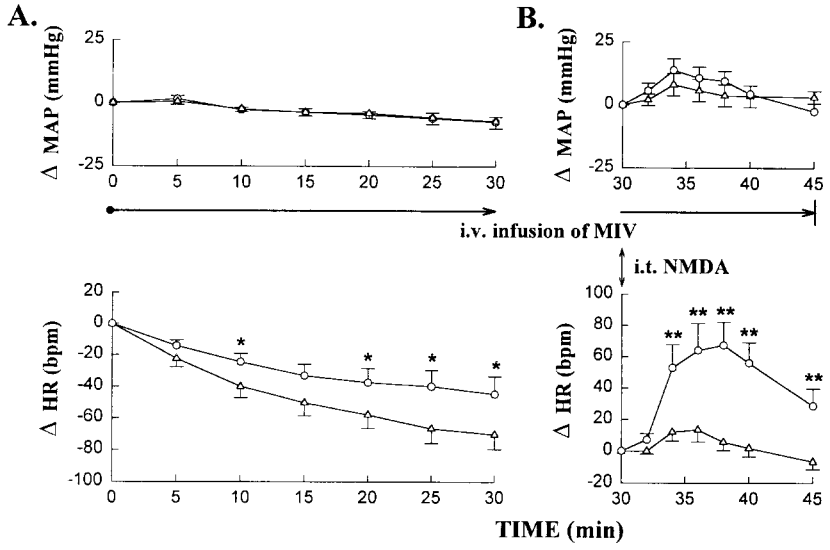

Fig. 2. (A) Changes in mean arterial pressure (MAP) and heart rate (HR) during i.v. infusion of mivazerol (MIV) in the yohimbine- or vehicle-pretreated rats. (B) Changes in mean arterial pressure and heart rate after i.t. injection of *N*-methyl-D-aspartic acid (NMDA) during i.v. infusion of mivazerol in the yohimbine- or vehicle-pretreated rats. Yohimbine (150 nmol/kg) or distilled water (10 μl) as a vehicle was administered i.t. at the T_2 level 10 min before the start (0 min) of i.v. infusion of mivazerol (15.3 μg/kg per h). NMDA (15 nmol/kg) was postinjected i.t. at the T_2 level 30 min after the start of i.v. infusion of mivazerol. Data are expressed as means ± S.E.M. Baseline values of mean arterial pressure (mmHg) and heart rate (bpm) at 0 min: 124.6 ± 6.0 and 418.7 ± 18.0 in the vehicle (i.t.)-pretreated group ($n = 7$, open triangles); 130.3 ± 6.7 and 427.0 ± 22.2 in the yohimbine (i.t.)-pretreated group ($n = 7$, open circles), * $P < 0.05$, ** $P < 0.01$ vs. vehicle (i.t.)-pretreated group.

per h; flow rate 0.1 ml/min) which was started from 10 min before i.v. infusion of mivazerol or vehicle (pentobarbital solution itself) until the end of the experiment (Fig. 1). Intravenous infusion of mivazerol (15.3 μg/kg per h) produced a significant decrease in heart rate but not in mean arterial pressure (Fig. 1A). The tachycardic response to i.t. injection of *N*-methyl-D-aspartate (NMDA) (15 nmol/kg) at the T_2 level was significantly attenuated by the i.v. infusion of mivazerol (Fig. 1B). The i.t. pretreatment with yohimbine (150 nmol/kg) at the T_2 level significantly attenuated the bradycardic response to i.v. infusion of mivazerol (15.3 μg/kg per h) (Fig. 2A) and blocked the effect of i.v. infusion of mivazerol (15.3 μg/kg per h) on the tachycardic response to i.t. injection of NMDA (15 nmol/kg) (Fig. 2B). In seven rats, i.v. administration of the same dose of yohimbine that was given intrathecally, had no effect on the bradycardic effect of i.v. infusion of mivazerol (15.3 μg/kg per h) after which changes in heart rate (beats per minute) were as follows: −19.0 ± 3.5 at 5 min, −36.4 ± 5.6 at 10 min, −51.4 ± 7.1 at 15 min, −59.1 ± 8.2 at 20 min, −67.6 ± 8.7 at 25 min and −74.6 ± 9.4 at 30 min.

4. Discussion

In the present study, i.v. infusion of mivazerol in the pentobarbital-anesthetized rat elicited a significant decrease in heart rate but not in mean arterial pressure. In addition, the bradycardic response to i.v. infusion of mivazerol was attenuated by prior i.t. injection of an α_2-adrenoceptor antagonist, yohimbine. These results are consistent with our previous findings that i.v. infusion of mivazerol did not significantly alter blood pressure but caused a dose-related decrease in heart rate, which was blocked by i.v. pretreatment with the α_2-adrenoceptor antagonist rauwolscine (Zhang et al., 1996). Classical α_2-adrenoceptor agonists such as clonidine and dexmedetomidine produce both hypotension and bradycardia (Kallio et al., 1989; Kobinger, 1978). Although reasons for the discrepancy of hemodynamic effects between mivazerol and other α_2-adrenoceptor agonists such as clonidine and dexmedetomidine are not apparent, it may be, in part, due to unique properties of mivazerol. For example, mivazerol only poorly penetrates the brain when infused i.v. and has higher α_2-adrenoceptor/imidazoline receptor selectivity than clonidine or dexmedetomidine (unpublished data).

Our main findings from the present study were that the tachycardic response to i.t. injection of NMDA at the T_2 level was significantly attenuated by an i.v. infusion of mivazerol and that this effect of mivazerol was blocked by i.t. pretreatment with yohimbine at the T_2 level. It has been, recently, reported that blood-brain barrier and blood-spinal cord barrier are not identical in their permeability characteristics (Banks et al., 1994) and that, in addition, the blood-spinal cord barrier was more permeable to some substances than the blood-brain barrier (Prockop et al., 1995). Mivazerol illustrates this case because autoradiographic studies showed that it was detected in spinal cord, but not in brain, after radiolabelled mivazerol was infused i.v. (E. Wülfert, unpublished data). Taken together, our results raise the possibility of a functional interaction

between spinal α_2-adrenoceptors and NMDA receptors in the control of heart rate. In concurrence with this argument, it has recently been shown that an increased potassium conductance is responsible for the hyperpolarization produced by α_2-adrenoceptors in sympathetic preganglionic neurons (Inokuchi et al., 1992). It has also been suggested that noradrenaline increases potassium conductance and reduces both NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptor-mediated neurotransmission via activation of α_2-adrenoceptors in the mouse entorhinal cortex (Pralong and Magistretti, 1995).

In summary, the present results suggest that the bradycardic effect due to i.v. infusion of mivazerol is mediated, at least in part, by the activation of α_2-adrenoceptors in upper thoracic spinal segments. Furthermore, we propose that there may be a functional interaction between spinal α_2-adrenoceptors as an inhibitory mechanism and NMDA receptors as a facilitatory mechanism, in the control of heart rate.

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