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Involvement of GABA and glutamate receptors in the blood pressure responses to intrathecally injected sodium nitroprusside in anesthetized rats

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Abstract

In pentobarbital-anesthetized rats the intrathecal (i.t.) injection of the nitric oxide (NO) donor, sodium nitroprusside (125, 250 and 500 nmol), induced a dose-dependent hypotensive response followed by a dose-dependent pressor effect. The pressor response to sodium nitroprusside (250 nmol) was reduced to 30% of the control value by the selective antagonist for AMPA/kainate receptors, 6,7-dinitroquinoxaline-2,3-dione (50 nmol, i.t.), whereas it was not modified by the selective NMDA receptor antagonist, 2-amino-5-phosphono-valeric acid (30 nmol, i.t.). The hypotensive effect of sodium nitroprusside was antagonized by the $GABA_A$ receptor antagonists, bicuculline (4.4 nmol, i.t.) and picrotoxin (4.4 nmol, i.t.), and also by the $GABA_B$ receptor antagonist, 2-hydroxy saclofen (113 nmol, i.t.). The blood pressure responses to sodium nitroprusside were not modified by blockade of muscarinic receptors with methyl atropine (164 nmol, i.t.), or of nicotinic receptors with hexamethonium (211 nmol, i.t.), of α_1-adrenoceptors with prazosin (3.1 nmol, i.t.), of α_2-adrenoceptors with yohimbine (2.8 μmol/kg, i.v.), of 5-HT receptors with methysergide (5.1 μmol/kg, i.v.), or of glycine receptors with strychnine (65 nmol, i.t.). It is concluded that NO generated from sodium nitroprusside in the spinal cord exerts inhibitory and excitatory effects on blood pressure probably through the release of GABA and glutamate, respectively. The inhibitory action on blood pressure involves the stimulation of spinal $GABA_A$ and $GABA_B$ receptors whereas the excitatory response to glutamate appears to be mediated through the activation of spinal AMPA/kainate receptors.

Keywords: Nitric oxide (NO); Sodium nitroprusside; Spinal cord; Blood pressure; Glutamate receptor, ionotropic; GABA receptor

1. Introduction

In vitro studies have shown that, in the central nervous system, nitric oxide (NO) enhances the spontaneous as well as the evoked release of several neurotransmitters such as glutamate (Lonart et al., 1992; Hirsch et al., 1993; Montague et al., 1994), noradrenaline (Lonart et al., 1992; Montague et al., 1994; Stout and Woodward, 1995), acetylcholine (Lonart et al., 1992) and γ-aminobutyric acid (GABA) (Lonart et al., 1992). Moreover, the administration of the NO donor, sodium nitroprusside, into the paraventricular nucleus of anesthetized rats increases the local release of excitatory as well as of inhibitory amino acids such as glutamate and GABA (Horn et al., 1994).

A hypotensive response followed by a pressor effect has previously been reported for intrathecally (i.t.) injected sodium nitroprusside in pentobarbital-anesthetized rats (García et al., 1997). Based on this result, it was proposed that NO generated from sodium nitroprusside in the spinal cord of anesthetized rats could increase the release of inhibitory as well as of excitatory neurotransmitters, which in turn might modify sympathetic preganglionic nerve activity (García et al., 1997). To further evaluate this hypothesis, in the present study we analyzed whether the effects of i.t. injection of sodium nitroprusside in pentobarbital-anesthetized rats were modified by selective blockade of receptors for glutamate, acetylcholine, 5-hydroxytryptamine, catecholamines, GABA and glycine.

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2. Materials and methods

2.1. Surgical procedures

Procedures for the evaluation of cardiovascular effects of i.t. injection of drugs were similar to those previously described (García et al., 1997). The animals were housed in groups of four in a room maintained at 21–23°C under a 12 h light–dark cycle. Food and water were freely available. Female Wistar rats (180–230 g) were anesthetized with sodium pentobarbital. The animals received an initial dose of sodium pentobarbital (40 mg/kg, i.p.), supplemented with 20 mg/kg (i.p.) before introduction of the i.t. catheter. An additional dose (20 mg/kg) was given during the experiment when necessary. The depth of anesthesia (surgical plane) was verified by the absence of the eyelid reflex. Although the animals breathed spontaneously, the trachea was cannulated to avoid respiratory disorders related to the accumulation of secretions in the superior airways. A polyethylene cannula was placed in the right femoral artery for recording of the blood pressure. In some experiments, a femoral vein was cannulated for i.v. injection of drugs. While the blood pressure was recorded, a cannula was positioned at the level of the T_{12}-L_1 intervertebral space as described by Dib (1984). Briefly, a cannula (outside diameter: 0.65 mm) was inserted into the subarachnoid space at the level of the C_8-T_1 vertebrae and gently pushed downward 4.5 cm. The position of the cannula was verified postmortem by opening the ventral aspect of the vertebrae to localize the cannula tip. The rectal temperature was maintained at 37–38°C by a heating lamp.

2.2. Blood pressure recording and heart rate calculation

Blood pressure was measured from the right femoral artery via a Statham P23 1D transducer and recorded on a Grass 7B polygraph (Quincy, MA, USA). The mean blood pressure was calculated from the formula: diastolic pressure $+1/3$ (systolic pressure $-$ diastolic pressure). The heart rate was calculated from the blood pressure record. Baseline blood pressure and heart rate were recorded for at least 30 min before the start of the experiment. Changes in mean blood pressure and heart rate induced by i.t. or i.v. injection of drugs refer to the differences between the recording made just before the beginning of the drug injection and the value at a given time.

2.3. Intrathecal injection of drugs

Drugs and saline solution were i.t. injected in volumes no greater than 4 μl, using a 10-μl Hamilton microsyringe. The rate of injection was 1 μl/min. For bolus i.v. administration of drugs the volume of injection was 0.1 ml and the cannula was flushed with 0.1 ml saline solution.

2.4. Experimental protocols

Doses of sodium nitroprusside of either 125 or 250 or 500 nmol were i.t. injected in a volume of 1 μl, in 1 min. The blood pressure and heart rate were recorded just before injection (time 0) and at the following times after the beginning of the injection: 1, 1.5, 2, 4, 6, 8, 10, 15, 20, 25 and 30 min. In some animals, sodium nitroprusside (250 nmol) was injected to animals receiving one of the following pretreatments or the corresponding vehicle solution: 2-amino-5-phosphonovaleric acid (APV; 30 nmol, i.t.), 6,7-dinitroquinoxaline-2,3-dione (DNQX, 50 nmol, i.t.), methysergide (5.1 μmol/kg, i.v.), prazosin (3.1 nmol, i.t.), yohimbine (2.8 μmol/kg, i.v.), methyl atropine (164 nmol, i.t.), hexamethonium (211 nmol, i.t.), bicuculline (4.4 nmol, i.t.), picrotoxin (4.4 nmol, i.t.), 2-hydroxy saclofen (113 nmol, i.t.), strychnine (65 nmol, i.t.). The period of time elapsed between the end of the i.t. injection of the antagonists and the beginning of the injection of sodium nitroprusside was 5 min. Methysergide and yohimbine were i.v. injected 5 and 60 min before sodium nitroprusside, respectively. Doses of antagonists were selected on the basis of results of previous studies (Timmermans et al., 1979; Ramirez et al., 1982; Hong et al., 1989; Hong and Henry, 1990; Aran and Hammond, 1991; Gradin et al., 1992; Khan et al., 1994a; García et al., 1996, 1997). Also, the effectiveness of antagonists to block the responses to the corresponding agonists was tested under our experimental conditions. The glycine receptor antagonist, strychnine, could not be tested because, in our hands, glycine had no effects on blood pressure when i.t. injected at the range of doses employed, from 50 to 200 nmol.

2.5. Drugs

Atropine methyl bromide, 2-amino-5-phosphonovaleric acid, bethanechol chloride, clonidine hydrochloride, 1,1-dimethyl-4-phenylpiperazinium iodide, 6,7-dinitroquinoxaline-2,3-dione, hexamethonium bromide, 5-hydroxytryptamine creatinine sulfate, *N*-methyl-D-aspartic acid, muscimol, noradrenaline bitartrate, picrotoxin, sodium nitroprusside and strychnine hydrochloride were purchased from Sigma (USA). Bicuculline methiodide, ($\pm$)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid hydrobromide and 2-hydroxy saclofen were purchased from RBI (USA). Baclofen was obtained from Ciba Geigy (Argentina). Yohimbine hydrochloride was obtained from Schering (Argentina). Methysergide bimaleate was obtained from Sandoz (Argentina). Prazosin hydrochloride was obtained from Pfizer (Argentina). 6,7-Dinitroquinoxaline-2,3-dione was dissolved in 0.75 M NaOH plus saline solution and the pH was adjusted to 7.8 with HCl. Prazosin, methysergide and 2-hydroxy saclofen were dissolved in 5% glucose. The remaining drugs were dissolved in saline solution. Drug doses refer to the respective free bases. For practical restrictions in the handling and preparation of the

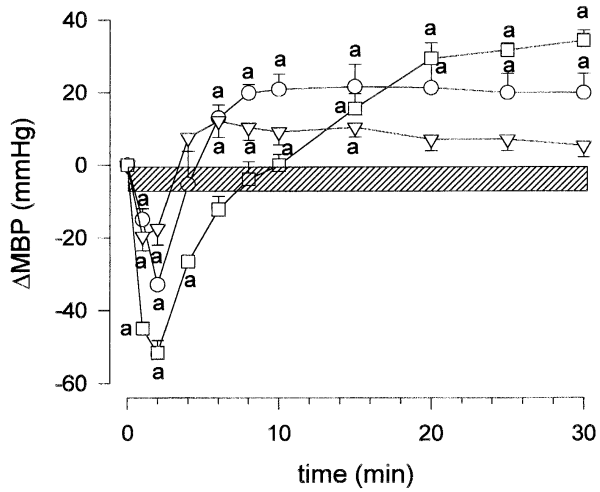

Fig. 1. Changes in mean blood pressure (ΔMBP, mmHg) induced by i.t. injection of sodium nitroprusside. Sodium nitroprusside (∇, 125 nmol; ○, 250 nmol; □, 500 nmol) was injected in 1 min, starting at time 0. The hatched bar represents the effect of i.t. injected saline solution. Shown are mean values ± S.E.M. for four to seven animals per group. [a] $P < 0.05$ vs. saline solution (analysis of variance followed by Newman–Keuls test). The resting MBP (mmHg)/HR (beats/min) values were: 100.3 ± 3.0/395 ± 11 for saline solution; 99.2 ± 6.3/407 ± 20 for 125 nmol sodium nitroprusside; 92.4 ± 4.5/398 ± 9 for 250 nmol sodium nitroprusside and 102.3 ± 3.3/410 ± 13 for 500 nmol sodium nitroprusside.

stock solutions, drug doses were expressed as μmol per kilogram of body weight for the i.v. injections and solely as nmol in the case of the i.t. administrations.

2.6. Statistics

All values represent the mean ± S.E.M. The statistical significance of differences was assessed either by Student's *t*-test or by one-factor analysis of variance followed by the Newman–Keuls' test. P values smaller than 0.05 were regarded as significant.

3. Results

3.1. Cardiovascular responses induced by intrathecal injection of sodium nitroprusside. Effects of excitatory and inhibitory amino acid receptor antagonists

The NO donor, sodium nitroprusside, at doses of 125, 250 and 500 nmol (i.t.) induced a dose-dependent decrease in the mean blood pressure that lasted approximately 2–4 min. This effect was followed by a dose-dependent long-lasting pressor response that persisted for at least 30 min (Fig. 1). Lower doses of sodium nitroprusside (15 to 90 nmol, i.t.) did not induce changes in the mean blood pressure (data not shown).The maximal change in heart rate induced by sodium nitroprusside (125 nmol: -7 ± 9 beats/min, $n = 4$; 250 nmol: -10 ± 9 beats/min, $n = 7$; 500 nmol: 13 ± 18 beats/min, $n = 5$) did not differ from the maximal change observed after i.t. injection of saline solution (4 ± 2 beats/min, $n = 5$).

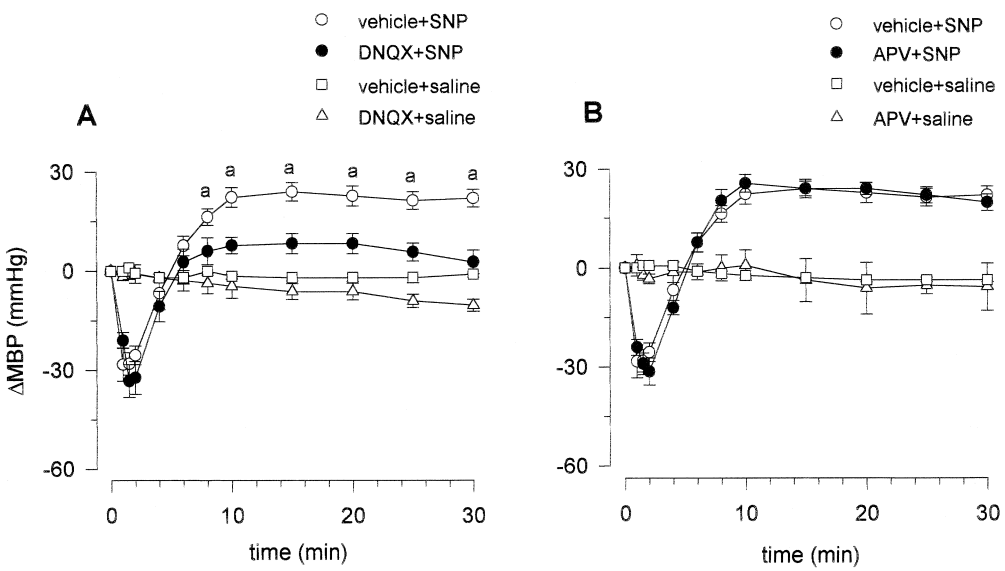

Fig. 2. Changes in mean blood pressure (ΔMBP, mmHg) induced by i.t. injection of sodium nitroprusside. Effects of glutamate receptors antagonists. Sodium nitroprusside (SNP: 250 nmol in 1 μl) or saline solution (1 μl) were i.t. injected in 1 min starting at time 0. Five min before time 0 the animals received either 6,7-dinitroquinoxaline-2,3-dione (DNQX: 50 nmol, i.t.), or 2-amino-5-phosphonovaleric acid (APV: 30 nmol, i.t.) or the corresponding vehicle solution for DNQX and APV. Shown are mean values ± S.E.M. for five to six animals per group. [a] $P < 0.01$ vs. DNQX plus SNP (analysis of variance followed by Newman–Keuls test). The resting mean blood pressure (mmHg)/heart rate (beats/min) values were: 98.7 ± 3.0/396 ± 18 for DNQX vehicle plus saline solution; 100.5 ± 2.5/389 ± 7 for APV vehicle plus saline solution; 103.9 ± 4.6/406 ± 13 for DNQX vehicle plus SNP; 98.1 ± 6.1/398 ± 11 for APV vehicle plus SNP; 99.6 ± 7.4/430 ± 15 for DNQX plus saline solution; 106.3 ± 2.3/426 ± 15 for APV plus saline solution; 97.0 ± 4.0/392 ± 19 for DNQX plus SNP; 100.7 ± 1.7/382 ± 11 for APV plus SNP.

As shown in Fig. 2A, a selective antagonist for ionotropic glutamate receptors of the AMPA/kainate subtype, the 6,7-dinitroquinoxaline-2,3-dione (DNQX), decreased by approximately 70% the pressor response to 250 nmol sodium nitroprusside. On the other hand, the hypertensive effect of sodium nitroprusside was not modified by 2-amino-5-phosphono-valeric acid (APV), a selective antagonist for ionotropic glutamate receptors of the NMDA subtype (Fig. 2B). Neither DNQX nor APV modified the transient hypotensive effect of sodium nitroprusside (Fig. 2A, B). The doses of the glutamate receptor antagonists employed for these experiments (50 nmol DNQX and 30 nmol APV; i.t.) entirely abolished the increases in blood pressure induced by i.t. injection of the corresponding specific agonists (Fig. 3A).

The $GABA_A$ receptor antagonists, bicuculline and picrotoxin, as well as the $GABA_B$ receptor antagonist, 2-hydroxy saclofen, at doses that antagonized the hypotensive responses induced by i.t. injection of the corresponding specific agonists (Fig. 3B), also reduced the hypotensive response to 250 nmol sodium nitroprusside (Fig. 4A, B and C). On the other hand, the hypotensive response to sodium nitroprusside was not modified by the glycine receptor antagonist, strychnine (65 nmol, i.t.) (Fig. 4D). The pressor response induced by i.t. injection of sodium nitroprusside was unmodified by either the GABA or the glycine receptor antagonists (Fig. 4).

With the exception of APV, that produced a slight but significant decrease in the baseline mean blood pressure, the different antagonists assayed modified neither heart rate nor blood pressure (Table 1).

3.2. Effects of antagonists for catecholamine receptors, acetylcholine receptors and 5-HT receptors on the cardiovascular responses induced by intrathecal injection of sodium nitroprusside

As shown in Table 2, neither the hypotensive nor the pressor responses to sodium nitroprusside were modified by blockade of muscarinic receptors with methyl atropine (164 nmol, i.t.), or of nicotinic receptors with hexamethonium (211 nmol, i.t.), of α_1-adrenoceptors with prazosin (3.1 nmol, i.t.), of α_2-adrenoceptors with yohimbine (2.8 μmol/kg, i.v.), or of 5-HT receptors with methysergide (5.1 μmol/kg, i.v.). The doses of antagonists assayed reduced the blood pressure responses induced by i.t. injection of the corresponding agonists (Fig. 3A, B).

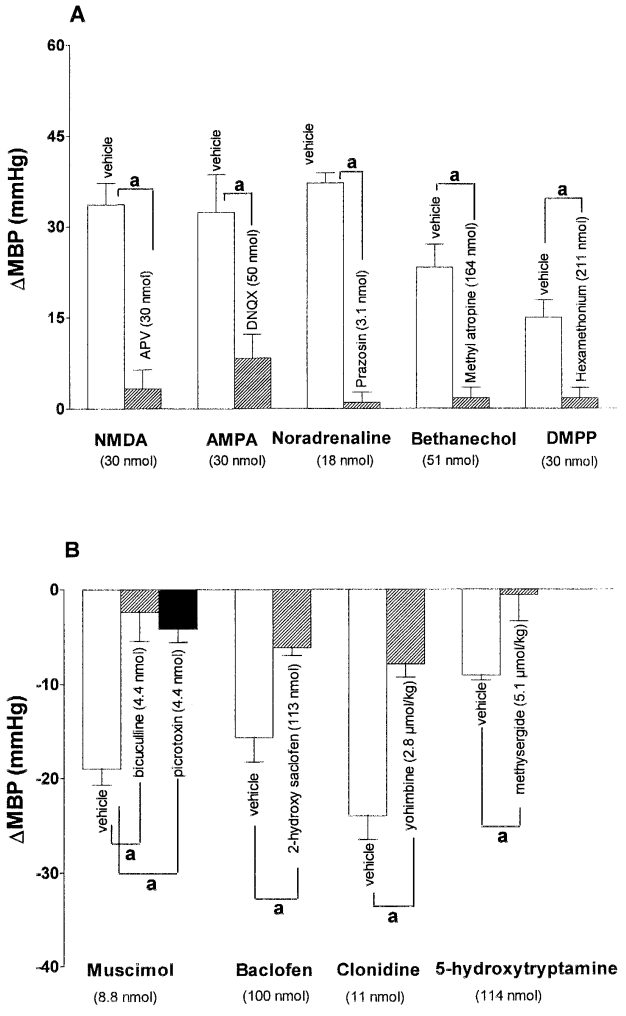

Fig. 3. Maximal changes in mean blood pressure (ΔMBP, mmHg) induced by i.t. injected NMDA, AMPA, noradrenaline, bethanechol, 1,1-dimethyl-4-phenylpiperazinium (DMPP), muscimol, baclofen, clonidine and 5-hydroxytryptamine. Effects of glutamate receptor, catecholamines receptor, cholinergic receptor, GABA receptor and 5-HT receptor antagonists. The agonists were i.t. injected in 1 min, starting at time 0. Intrathecal injection of either the antagonists or the corresponding vehicle solutions (5% glucose for prazosin and 2-hydroxy saclofen; saline solution for the remaining drugs) was performed 5 min before the beginning of the i.t. injection of the agonists. Intravenous injection of methysergide and yohimbine were performed 5 min or 60 min prior to clonidine or 5-hydroxytryptamine, respectively. Shown are mean values ± S.E.M. for four to seven animals per group. [a] $P < 0.05$ vs. the corresponding value in the vehicle-pretreated group (Student's *t*-test). The resting mean blood pressure (mmHg)/heart rate (beats/min) values were: 92.3 ± 6.0/406 ± 15 for APV vehicle plus NMDA; 107.0 ± 6.4/414 ± 13 for APV plus NMDA; 92.5 ± 3.4/394 ± 9 for DNQX vehicle plus AMPA; 107.3 ± 2.6/402 ± 13 for DNQX plus AMPA; 107.8 ± 2.5/390 ± 7 for prazosin vehicle plus noradrenaline; 101.7 ± 1.0/387 ± 3 for prazosin plus noradrenaline; 104.4 ± 2.0/385 ± 3 for methyl atropine vehicle plus bethanechol; 98.7 ± 2.1/380 ± 8 for methyl atropine plus bethanechol; 100.6 ± 4.7/398 ± 10 for hexamethonium vehicle plus DMPP; 103.3 ± 5.1/389 ± 15 for hexamethonium plus DMPP; 102.3 ± 5.0/410 ± 13 for bicuculline/picrotoxin vehicle plus muscimol; 95.0 ± 3.4/400 ± 10 for bicuculline plus muscimol; 91.7 ± 7.0/395 ± 7 for picrotoxin plus muscimol; 105.2 ± 3.7/390 ± 8 for 2-hydroxy saclofen vehicle plus baclofen; 98.7 ± 2.1/410 ± 11 for 2-hydroxy saclofen plus baclofen; 108.0 ± 2.5/380 ± 7 for yohimbine vehicle plus clonidine; 99. 6 ± 3.1/382 ± 9 for yohimbine plus clonidine; 103.3 ± 4.5/388 ± 12 for methysergide vehicle plus 5-hydroxytryptamine; 95.4 ± 6.0/390 ± 10 for methysergide plus 5-hydroxytryptamine.

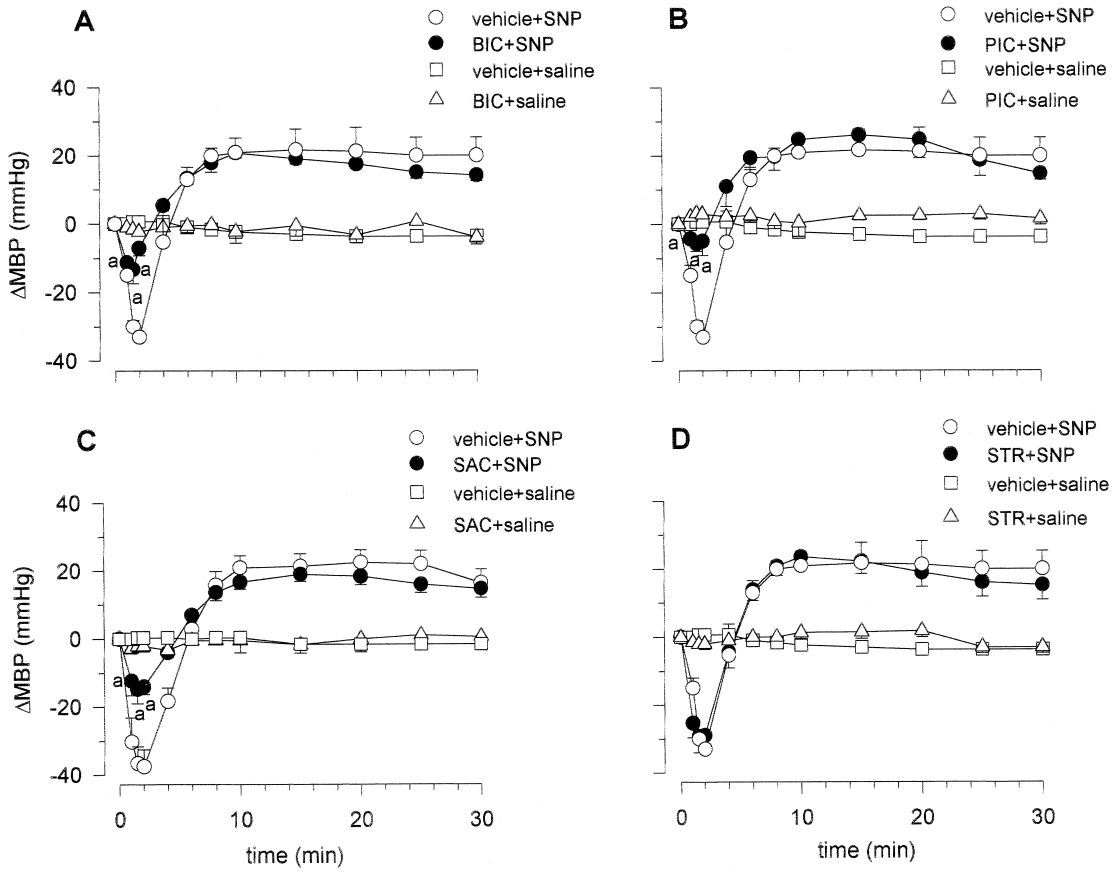

Fig. 4. Changes in mean blood pressure (ΔMBP, mmHg) induced by i.t. injection of sodium nitroprusside. Effects of GABA- and glycine-receptor antagonists. Sodium nitroprusside (SNP: 250 nmol in 1 μl) or saline solution (1 μl) were i.t. injected in 1 min starting at time 0. Five min before time 0 the animals received either picrotoxin (PIC: 4.4 nmol, i.t.), or bicuculline (BIC: 4.4 nmol, i.t.), or 2-hydroxy saclofen (SAC: 113 nmol, i.t.), or strychnine (STR: 65 nmol, i.t.), or the corresponding vehicle solution ((A), (B) and (D) saline solution; (C) 5% glucose). Shown are mean values ± S.E.M. for five to six animals per group. [a] $P < 0.01$ vs. the corresponding antagonist plus SNP (analysis of variance followed by Newman–Keuls test). The resting mean blood pressure (mmHg)/heart rate (beats/min) values were: 100.5 ± 2.5/389 ± 7 for vehicle in (A), (B) and (D) plus saline solution; 101.3 ± 3.6/404 ± 7 for vehicle in (C) plus saline solution; 104.7 ± 3.9/412 ± 13 for BIC plus saline solution; 93.3 ± 14.3/407 ± 22 for PIC plus saline solution; 96.7 ± 7.7/383 ± 9 for SAC plus saline solution; 97.1 ± 2.9/385 ± 6 for STR plus saline solution; 98.1 ± 6.9/398 ± 11 for saline solution plus SNP; 92.2 ± 6.5/378 ± 11 for 5% glucose plus SNP; 97.9 ± 4.4/397 ± 15 for BIC plus SNP; 101.3 ± 4.3/400 ± 16 for PIC plus SNP; 97.3 ± 4.2/398 ± 12 for SAC plus SNP; 102.5 ± 3.4/400 ± 8 for STR plus SNP.

Table 1
Mean blood pressure (MBP) and heart rate (HR) after injection of different receptor antagonists

Drug	Dose	*n*	MBP (mmHg)		HR (beats/min)	
			Before[a]	After[b]	Before[a]	After[b]
DNQX	50 nmol (i.t.)	9	98.1 ± 3.7	99.2 ± 4.0	406 ± 13	402 ± 13
APV	30 nmol (i.t.)	12	104.9 ± 3.1	98.4 ± 4.0 [c]	382 ± 11	384 ± 11
Bicuculline	4.4 nmol (i.t.)	9	101.7 ± 3.0	105.5 ± 2.8	406 ± 10	410 ± 9
Picrotoxin	4.4 nmol (i.t.)	9	97.8 ± 6.4	98.5 ± 7.0	403 ± 21	404 ± 23
2-Hidroxy saclofen	113 nmol (i.t.)	8	97.1 ± 6.3	89.0 ± 11.0	390 ± 12	390 ± 3
Methyl atropine	164 nmol (i.t.)	9	105.3 ± 4.6	107.3 ± 5.6	394 ± 10	386 ± 7
Hexamethonium	211 nmol (i.t.)	9	104.7 ± 3.3	102.3 ± 3.3	396 ± 17	400 ± 21
Strychnine	65 nmol (i.t.)	9	102.5 ± 3.4	106.4 ± 4.6	400 ± 8	392 ± 9
Yohimbine	2.8 μmol/kg (i.v.)	5	107.3 ± 4.1	102.7 ± 3.2	376 ± 19	364 ± 24
Methysergide	5.1 μmol/kg (i.v.)	6	113.3 ± 5.9	111.1 ± 6.3	392 ± 14	373 ± 11
Prazosin	3.1 nmol (i.t.)	9	91.9 ± 4.8	92.4 ± 4.7	390 ± 6	391 ± 7

[a] Mean blood pressure (MBP) and heart rate (HR) determined before either i.t. or i.v. injection of the corresponding drugs.
[b] MBP and HR determined either 60 min after the injection of yohimbine or 5 min after the injection of the remaining drugs. Shown are mean values ± S.E.M. *n* = number of experiments.
[c] $P < 0.05$ vs. the corresponding controls before drug injection (Student *t*-test for paired values).

Table 2
Lack of effects of antagonists for acetylcholine, catecholamines and 5-HT receptors on the maximal hypotensive and on the maximal pressor responses induced by intrathecal injection of sodium nitroprusside (SNP)

Pretreatment	*n*	Maximal decrease in MBP (mmHg) induced by SNP	Maximal increase in MBP (mmHg) induced by SNP
Vehicle (saline solution, i.t.)	7	-33.0 ± 1.3	20.9 ± 4.3
Methyl atropine (164 nmol, i.t.)	5	-28.0 ± 2.4	21.7 ± 2.3
Hexamethonium (211 nmol, i.t.)	5	-31.3 ± 5.1	24.7 ± 3.1
Methysergide (5.1 μmol/kg, i.v.)	6	-24.1 ± 8.2	18.6 ± 3.3
Yohimbine (2.8 μmol/kg, i.v.)	5	-40.7 ± 11.5	19.2 ± 3.6
Vehicle (5% glucose, i.t.)	6	-37.5 ± 5.0	22.5 ± 3.7
Prazosin (3.1 nmol, i.t.)	6	-45.0 ± 8.9	20.5 ± 3.3

Sodium nitroprusside (250 nmol in 1 μl) was i.t. injected in 1 min starting at time 0. Intrathecal injection of either the antagonists or the corresponding vehicle solutions (5% glucose for prazosin and saline solution for the remaining drugs) was performed 5 min before the beginning of the i.t. injection of sodium nitroprusside. Intravenous injection of methysergide and yohimbine was performed 5 min or 60 min prior to sodium nitroprusside, respectively. Shown are mean values ± S.E.M. n = number of experiments.

On the other hand, the antagonists themselves did not modify either resting mean blood pressure or baseline heart rate (Table 1).

4. Discussion

L-Glutamate, or a related amino acid, is likely to be the major excitatory neurotransmitter at preganglionic sympathetic neurons (Bazil and Gordon, 1991; Chalmers et al., 1992, 1994; West and Huang, 1994). The actions of excitatory amino acids on these neurons depend on the activation of NMDA as well as non-NMDA (AMPA/kainate) ionotropic receptors in the spinal cord (Shen et al., 1990; Sundaram and Sapru, 1991; Hong and Henry, 1992a,b; West and Huang, 1994; Krupp and Feltz, 1995). The present results showed that the AMPA/kainate receptor antagonist, DNQX, reduces the enhancement in the blood pressure induced by sodium nitroprusside whereas APV, a specific NMDA receptor antagonist, does not modify this response. Hence, the pressor effect of i.t. administered sodium nitroprusside could be related to the release of glutamate which, in turn, activates selectively AMPA/kainate receptors in the spinal cord. In support of the possible participation of excitatory amino acids in the pressor response to sodium nitroprusside at spinal level, there is evidence that endogenous NO as well as NO generated from NO donors induces the release of glutamate and other excitatory amino acids in the central nervous system (Lonart et al., 1992; Hirsch et al., 1993; Horn et al., 1994; Montague et al., 1994).

Results of several studies suggest that NMDA and non-NMDA receptors influencing preganglionic sympathetic neuron activity could be differentially activated by separate spinal pathways (Shen et al., 1990; West and Huang, 1994; Krupp and Feltz, 1995). Therefore, a possible interpretation of our results with sodium nitroprusside in DNQX- and APV-treated rats is that NO generated from sodium nitroprusside may facilitate the release of excitatory amino acids from neuronal pathways linked to preganglionic sympathetic neurons through the activation of spinal non-NMDA receptors. It is of interest to note that, at least in our hands, endogenous NO does not produce a hypertensive response and its synthesis in the rat spinal cord appears to be tonically activated through the stimulation of NMDA receptors (García et al., 1997).

Although preganglionic sympathetic neurons receive an excitatory input from catecholaminergic bulbospinal pathways (Ross et al., 1984; Chalmers and Pilowsky, 1991) and the i.t. injection of cholinergic agonists induces increases in the blood pressure through the activation of muscarinic and nicotinic receptors in the spinal cord (Marshall and Buccafusco, 1987; Khan et al., 1994b), neither catecholamines nor acetylcholine appear to be involved in the hypertensive effect of i.t. administered sodium nitroprusside. This is based on the fact that the pressor response to sodium nitroprusside remained unchanged after α_1-adrenoceptor, muscarinic and nicotinic receptor blockade in the spinal cord. Preganglionic sympathetic nerve activity is enhanced by several other substances such as angiotensin II and substance P (Coote, 1988). The participation of these neurotransmitters/neuromodulators in the pressor response to i.t. injection of sodium nitroprusside remains to be investigated.

There is evidence that, in the spinal cord, GABA inhibits sympathetic preganglionic neuron activity (Backman and Henry, 1983; Wu and Dun, 1993) and induces a decrease in both blood pressure and heart rate (Gordon, 1985; Hasséssian et al., 1991). Moreover, there is evidence of synaptic contacts between GABAergic nerve terminals and sympathetic preganglionic neurons (Bacon and Smith, 1988; Chalmers et al., 1992). The inhibitory cardiovascular effects of GABA in the spinal cord are mediated by the activation of both $GABA_A$ and $GABA_B$ spinal receptors (Gordon, 1985; Hasséssian et al., 1991; Hong and Henry, 1991). Therefore, the finding that $GABA_A$ receptor-blocking drugs as well as a $GABA_B$ receptor antagonist antago-

nized the hypotensive response to sodium nitroprusside could suggest that this effect is mediated by the release of GABA and its subsequent inhibitory action on the sympathetic preganglionic outflow. In support of this view, there is evidence that NO and NO donors enhance the release of GABA in the central nervous system (Lonart et al., 1992; Horn et al., 1994; Segovia et al., 1994). Moreover, Wu and Dun (1996) have shown that NO exhibits facilitatory effects on inhibitory inputs to sympathetic preganglionic neurons in slice preparations of neonatal rats.

Glycine, another amino acid with inhibitory actions on sympathetic preganglionic neurons (Backman and Henry, 1983; Mo and Dun, 1987; Wu and Dun, 1993), does not appear to be involved in the hypotensive response to i.t. injected sodium nitroprusside, at least because the glycine receptor antagonist, strychnine, did not modify the response. It should be noticed, however, that we did not use strychnine at doses higher than 65 nmol because preliminary experiments showed that it produces convulsions when i.t. injected to anesthetized rats.

In vitro as well as in vivo studies have shown that the activation of spinal α_2-adrenoceptors by catecholamines induces a decrease in sympathetic preganglionic neuron activity, in blood pressure and in heart rate (Miyazaki et al., 1989; Sundaram et al., 1991; Gradin et al., 1992; Malhotra et al., 1993; García et al., 1996). It is unlikely that, in the present study, the α_2-inhibitory action of spinal catecholamines participated in the hypotensive effect of sodium nitroprusside because this response remained unchanged after pretreatment with the α_2-adrenoceptor antagonist, yohimbine.

The hypotension caused by i.t. injection of sodium nitroprusside is apparently unrelated to an action of 5-hydroxytryptamine in the spinal cord because the 5-HT receptor antagonist, methysergide, failed to alter this response at a dose that reduced the hypotensive effect of i.t. injected 5-hydroxytryptamine.

The possibility of a peripheral vasorelaxant effect of 250 nmol sodium nitroprusside as a consequence of its leakage from the site of injection cannot be disregarded. This is because it was found that nicotinic ganglionic blockade does not entirely prevent the hypotensive effect of i.t. injected sodium nitroprusside (García et al., 1997).

Whereas the observation that the decrease in the baseline mean blood pressure produced by blockade of NMDA receptors by APV could suggest tonic activation of preganglionic sympathetic neurons through the stimulation of NMDA receptors by glutamate, the opposite observation, i.e. the lack of effect of the other receptor antagonists employed, cannot exclude the participation of either AMPA/kainate receptors or receptors for neurotransmitters such as catecholamines, GABA and 5-hydroxytryptamine in the control of the resting mean blood pressure. This is mainly because single doses of antagonists were employed. Further experiments with different doses of the antagonists would contribute to elucidation of this matter.

5. Conclusions

In conclusion, the results suggest that, in the spinal cord of pentobarbital-anesthetized rats, NO generated from SNP exerts inhibitory and excitatory effects on blood pressure, probably through the release of GABA and glutamate, respectively. The inhibitory action on blood pressure involves the stimulation of spinal $GABA_A$ and $GABA_B$ receptors whereas the excitatory response appears to be mediated through the activation of spinal AMPA/kainate receptors.

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