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Prevention by NMDA receptor antagonists of the centrally-evoked increases of cardiac inotropic responses in rabbits

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1 The purpose of this study was to investigate further the role of the excitatory amino acid (EAA) system of neurotransmission, particularly of the NMDA receptor, in the central regulation of cardiac function.

2 Electrical stimulation of the paraventricular nucleus of the hypothalamus (PVN) in pentobarbitone anaesthetized rabbits induced a cardiovascular response mainly characterized by a positive inotropic effect, hypertension and a marked increase in the myocardial oxygen demand index.

3 The intracerebroventricular (i.c.v.) or intravenous (i.v.) injection of different EAA antagonists acting on different sites of the NMDA receptor/channel complex dose-dependently blunted the excitatory cardiovascular effects of PVN stimulation.

4 5,7 Dichlorokynurenic acid was used as a specific glycine site antagonist and 2-amino-5-phosphonovaleric acid was used to block the agonist recognition site; ketamine was used as a channel blocker site antagonist and ifenprodil as a blocker of the polyamine binding site.

5 5,7 Dichlorokynurenic acid (125 and 250 $\mu g\,kg^{-1}$, i.c.v.) virtually abolished the cardiovascular responses, inducing only haemodynamic depression at the highest dose used. 2-Amino-5-phosphonovaleric acid (0.1 to 1.0 $mg\,kg^{-1}$, i.c.v.) elicited a reduction of the peak values observed during PVN stimulation which was accompanied by a decrease of the basal cardiovascular parameters. Ketamine (2.5 and 10 $mg\,kg^{-1}$) and ifenprodil (1 $mg\,kg^{-1}$), injected intravenously, blocked the haemodynamic response induced by PVN stimulation without marked reduction of the basal haemodynamics.

6 It is concluded that glutamate neurotransmission is not only involved in vasomotor tone control but also in the central control of cardiac function and can therefore modulate the myocardial oxygen demand.

Keywords: Glutamate; NMDA antagonists; CNS; PVN stimulation; haemodynamic responses

Introduction

It is now generally accepted that excitatory amino acids (EAA), mainly glutamate, are important and ubiquitous excitatory neurotransmitters in the mammalian central nervous system and are involved in the mediation of physiological functions such as learning, memory, synaptic plasticity and cardiovascular regulation (Collingridge & Lester, 1989; Monaghan *et al.*, 1989; Young & Fagg, 1990). Moreover, EAA receptors are known to mediate synaptic excitations throughout the central nervous system (Headley & Grillner, 1990). The role of glutamatergic neurotransmission in the central regulation of cardiovascular function is emerging. There is increasing evidence for the involvement of EAA in the modulation of the baroreceptor reflex and of vasomotor tone (Cotman *et al.*, 1987; Guyenet *et al.*, 1987; Kubo & Kihara, 1988; Le Galloudec *et al.*, 1989). This system can modulate the activity of the vasomotor bulbospinal neurones of the ventrolateral medulla (Kubo *et al.*, 1986; Kihara *et al.*, 1989; Kao *et al.*, 1991; Chalmers & Pilowsky, 1991) which in turn modulates the activity of the sympathetic preganglionic neurones of the intermediolateral cell column in the spinal cord (Mills *et al.*, 1988; 1990). In this context, it has been recently reported that the intrathecal administration of glutamate antagonists in normotensive rats can attenuate the hypertensive response evoked by electrical stimulation of the rostral ventrolateral medulla, without affecting the resting levels of the cardiovascular parameters (Mills *et al.*, 1988). Moreover, the glutamatergic system of neurotransmission seems to be involved in the pathophysiology of arterial hypertension in genetically hypertensive rats (Mills *et al.*, 1990).

In the present work, we focused our attention on the involvement of glutamatergic neurotransmission in the modulation of cardiac inotropism which is one of the most important components of myocardial oxygen demand (MOD).

It had been shown previously that the activation of different brain regions, especially hypothalamic, could induce not only excitatory haemodynamic responses but also myocardial ischaemia and ventricular fibrillation (Lown *et al.*, 1977). For instance, Azevedo *et al.* (1980) reported that electrical stimulation of the paraventricular nucleus of the hypothalamus (PVN) in anaesthetized rabbits induced excitatory cardiovascular responses mainly characterized by important increases in cardiac output.

We used an experimental model of electrical stimulation of the PVN in the rabbit, which markedly increased arterial pressure and cardiac inotropic state, and studied the ability of NMDA receptor antagonists to modulate these responses.

Methods

Animals and haemodynamic measurements

Normotensive male rabbits (Zika strain) weighing between 2.5 and 3.5 kg were anaesthetized with 40 $mg\,kg^{-1}$ sodium

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pentobarbitone injected through the marginal vein of the ear; anaesthesia was complemented by another i.v. injection of pentobarbitone of 5 mg kg^{-1} immediately before the beginning of control electrical stimulations. Rectal temperature was maintained at 38 ± 0.5°C with the aid of a warming blanket as soon as anaesthesia was established (Harvard Apparatus LTD, Millis, MA, U.S.A.). The animals were tracheotomized, immobilized with pancuronium bromide (1 mg kg^{-1}, i.v.) and artificially ventilated with room air (Hugo Sachs Elektronic model 6025, March-Hugstetten, Germany). The ventilation parameters were adjusted to maintain PaO_2 at approximately 100 mgHg and $PaCO_2$ below 40 mmHg.

The right femoral vein was catheterized to permit i.v. injections and the instantaneous arterial pressure was measured through a catheter placed in the abdominal aorta via the right femoral artery connected to a Statham P23 Db transducer, which was in turn connected to a pressure processor and recorder (Gould Electronics model BS-272, Longjumeau, France). Mean arterial pressure (MAP) was calculated as diastolic pressure plus one third of the differential pressure (Ganong, 1971). Heart rate was also continuously monitored from the pressure signal with a Gould Biotach amplifier (model 13-4615-66). Left ventricular pressure and the maximum rate of rise of left ventricular pressure (dP/dt_{max}) were measured with a micromanometer tipped catheter (Gaeltec LTD, model ICT/B, Dunvegan, U.K.) placed in the left ventricle via the right carotid artery. The dP/dt_{max} was obtained with a Philips differentiator model 133-1-4331 (Bern, Switzerland). The myocardial oxygen demand was evaluated by the 'triple product' index (Baller *et al.*, 1979). This index was calculated by multiplying the heart rate by the systolic blood pressure and the maximum rate of rise in left ventricular pressure divided by 10^6 to reduce it to convenient units. This product will be referred to subsequently as the 'triple product'.

Electrical stimulation of the PVN

The head of the animal was placed in a stereotaxic apparatus (Unimécanique, Epinay/Seine, France). A craniotomy was performed and the dura mater drilled to permit stereotaxic placement of the concentric bipolar stainless steel electrode of 0.1 mm diameter (Rhodes Medical, model SNE-100). The stimulus consisted of a 5-s train of 1 ms rectangular pulses at a frequency of 100 Hz. In preliminary experiments, increasing current intensities ranging from 50 to 500 μA (Hugo Sachs T stimulator and a type 251 constant current unit, March-Hugstetten, Germany) were applied in order to establish intensity-effect relationships in our experimental model. We observed intensity-related increases in dP/dt_{max} until 500 μA, when a plateau was attained. Higher intensities induced cardiac rhythm disturbances without further increases in dP/dt_{max}. We then used current intensities ranging from 100 to 300 μA, inducing dP/dt_{max} increases of about 30%. The sites of stimulation were located according to the atlas of Sawyer *et al.* (1954) and the following coordinates were used: AP = from + 0.5 to 1.0 mm from bregma; L = from 0.5 to 1 mm and V = from − 12 to − 13 mm.

Intracerebral injections

A second craniotomy was performed to inject drugs into the left lateral ventricle. Normal saline solution (control group) or glutamate antagonists which do not cross the blood-brain barrier were injected in a constant volume of 100 μl of saline solution by use of a Hamilton micro-syringe (Hamilton Bonaduz AG, Switzerland) in the following stereotaxic coordinates: AP = − 4.5 mm from bregma; L = 8 mm and 6 mm down from the cranial surface (Sawyer *et al.*, 1954).

Experimental procedure

The electrodes were inserted stereotaxically in the PVN and at least three electrical stimulations at 5 min intervals inducing similar rises in dP/dt_{max} were performed and averaged before drug injections. When the haemodynamic responses obtained varied by less than 10% between stimulations, the response was considered to be stable and the drugs were injected either i.v. in 500 μl or i.c.v. in 100 μl normal saline solution followed by electrical stimulation 5 min later.

Location of sites of stimulation

At the end of the experiments to determine the location of the hypothalamic area where the electrical stimulation provoked reproducible significant increases of dP/dt_{max}, electrolytic lesions were made by a current of 1 mA for 10 s. After the animals were killed, their brains were fixed *in situ* by perfusion of a 10% formol saline solution through the carotid artery, then removed and conserved in 10% formol saline before embedding in paraffin Serial sections (10 μm) were cut and stained with haematoxyllin-eosin. The electrode position was located by use of the atlas of Sawyer *et al.* (1954).

Drugs

The following drugs were used: sodium pentobarbitone (Nembutal, Abbott Lab., North Chicago, IL, U.S.A.); pancuronium bromide (Pavulon, Organon Tecknica, Fresnes, France); 5-7 dichloro-kynurenic acid (Tocris Neuramin, England); ifenprodil (Vadilex, Synthélabo, France); ketamine (Ketalar, Parke Davis, France); 2-amino-5-phosphonovaleric acid (Tocris Neuramin, England).

Statistical analysis

All results are expressed as means ± s.e.mean. The effects of the treatments on baseline haemodynamics and on the responses to electrical stimulation were analysed by repeated ANOVA measures followed by Scheffe's test to determine statistically significant differences. Comparisons between the control group (saline-injected) and treated groups (animals injected with antagonists in saline solution) were made by one way ANOVA. All calculations were made by computer-assisted analyses with the Statview II programme (Abacus Concepts, Inc, Berkeley, U.S.A.).

Results

Control experiments

The repetitive intracerebroventricular injections of saline solution (100 μl) did not induce significant changes in the baseline haemodynamic parameters or alterations in the cardiovascular response to electrical stimulation of the PVN throughout the period of the experiment (Table 1).

Haemodynamic effects of electrical stimulation of the PVN region

Electrical stimulation of the PVN in the pentobarbitone-anaesthetized rabbit induced significant increases in dP/dt_{max} (Table 1) which began immediately after stimulation was started and ended when it ceased, reaching a maximum of + 35 ± 2% above the baseline value ($n = 6$, $P < 0.01$). Neither saline injected i.c.v. nor the time (up to 60 min) influenced this response. There were also increases in mean arterial pressure reaching a maximum of + 42 ± 5% ($n = 6$, $P < 0.01$) when compared to the initial values of 101 ± 5 mmHg. The positive inotropic and hypertensive responses were accompanied by mild bradycardia during the first 2 stimulation periods and, when repeated, increased to reach a maximum of − 19 ± 9%; $n = 6$, $P < 0.05$ (Table 1). These positive inotropic and vasopressive responses led to marked

increases in the triple product index of myocardial oxygen demand, which increased regularly by about 60%.

Effects of i.c.v. injection of NMDA antagonists on the haemodynamic responses to electrical stimulation of the PVN

5,7 Dichloro-kynurenic acid (5,7 diCl-KYNA) The marked increases in dP/dt_{max} induced by electrical stimulation of the PVN were inhibited by prior i.c.v. injections of 125 and 250 $\mu g\,kg^{-1}$ of 5,7 diCl-KYNA in a dose-related manner. The arterial pressure followed the same pattern of response (Table 2). In this series of experiments, 5,7 diCl-KYNA produced a dose-related inhibition of the increase in the triple product elicited by electrical stimulation of the PVN (Figure 1).

2-amino-5-phosphonovaleric acid (APV) The i.c.v. injection of increasing doses of APV (0.1, 0.3 and 1 $mg\,kg^{-1}$) reduced dose-dependently the peak values of the cardiovascular parameters observed during the electrical stimulation of the PVN. This effect was mainly obtained by decreasing the basal haemodynamics (Table 3, Figure 2).

Effects of the intravenous injection of NMDA antagonists on the haemodynamic responses to electrical stimulation of the PVN

Ifenprodil The i.v. injection of 1 $mg\,kg^{-1}$ of ifenprodil reduced the response observed during PVN stimulation for all cardiovascular parameters (Figure 3). The triple product (TP) response decreased from 178 ± 18 to 131 ± 9 ($P<0.05$). In this series of experiments, the basal level of the TP was slightly reduced by ifenprodil while the basal value of dP/dt_{max} was not significantly changed (Table 4).

Ketamine The i.v. administration of ketamine (2.5 and 10 $mg\,kg^{-1}$) dose-dependently reduced the peak values obtained during PVN stimulation of all cardiovascular parameters. All the stimulation-induced responses were abolished by the highest dose of 10 $mg\,kg^{-1}$. In these two groups of experiments, ketamine never significantly modified the basal values of the cardiovascular parameters (Table 5, Figure 4).

Table 1 Control and peak values of the cardiovascular parameters observed upon electrical stimulation of the PVN of the anaesthetized rabbit before and after repetitive i.c.v. injection of 100 µl of isotonic saline solution (control experiments)

	Saline (number of i.c.v. injection)							
	Control values		1		2		3	
	C	*S*	*C*	*S*	*C*	*S*	*C*	*S*
dP/dt_{max} ($mmHg\,s^{-1}$)	2960 ± 240	3975 ± 284	2920 ± 224	3970 ± 286	2980 ± 286	3830 ± 414	3150 ± 250	4070 ± 303
SBP (mmHg)	123 ± 6	162 ± 11	121 ± 6	166 ± 11	123 ± 6	164 ± 10	124 ± 6	165 ± 10
DBP (mmHg)	90 ± 5	132 ± 10	89 ± 5	131 ± 9	90 ± 5	133 ± 9	91 ± 6	135 ± 10
MBP (mmHg)	101 ± 5	142 ± 10	100 ± 6	142 ± 10	101 ± 6	145 ± 4	102 ± 6	145 ± 10
HR (beats min^{-1})	278 ± 10	251 ± 12	272 ± 12	254 ± 9	276 ± 9	230 ± 16	272 ± 12	216 ± 20
TP ($mmHg^2\,s^{-1}$ b.p.m. 10^{-6})	102 ± 12	162 ± 19	96 ± 12	163 ± 20	100 ± 13	150 ± 22	106 ± 12	144 ± 23

The excitatory cardiovascular responses to electrical stimulation were highly reproducible over time (60 min). Data are represented as means ± s.e.mean of 6 experiments. (ANOVA for repeated measures). SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; HR, heart rate; TP, triple product; C, baseline values, S, electrical stimulation

Table 2 Effects of the cumulative intracerebroventricular administrations of 5,7 di-chloro kynurenic acid on basal haemodynamics and on the cardiovascular response to electrical stimulation of the PVN of the pentobarbitone anaesthetized rabbit

	Before treatment		*5,7 di-chloro Kynurenic acid* 125 $\mu g\,kg^{-1}$, *i.c.v.*		250 $\mu g\,kg^{-1}$, *i.c.v.*	
	C	*S*	*C*	*S*	*C*	*S*
dP/dt_{max} ($mmHg\,s^{-1}$)	4673 ± 486	6260 ± 248	4060 ± 380	5300 ± 141*	3700 ± 591†	4120 ± 668**
SBP (mmHg)	108 ± 9	145 ± 6	96 ± 9	119 ± 6*	93 ± 14	100 ± 14**
DBP (mmHg)	83 ± 7	125 ± 5	72 ± 9	99 ± 5*	71 ± 12	82 ± 13*
MBP (mmHg)	91 ± 7	131 ± 5	80 ± 9	106 ± 5*	78 ± 13	88 ± 14*
HR (beats min^{-1})	275 ± 10	253 ± 9	253 ± 9	246 ± 12NS	244 ± 7	250 ± 9NS
TP ($mmHg^2\,s^{-1}$ b.p.m. 10^{-6})	143 ± 23	211 ± 9	104 ± 18	156 ± 14*	93 ± 26†	115 ± 29**

C = baseline values; S = electrical stimulation. Each value represents the mean ± s.e.mean of 5 experiments. *$P<0.05$; **$P<0.01$ statistically significant effect of electrical stimulation when compared to values during stimulation before treatment; †$P<0.05$; significant effect of the treatment on basal cardiovascular parameters when compared to control values before treatment. (ANOVA for repeated measures followed by Scheffé's test). Abbreviations as in Table 1.

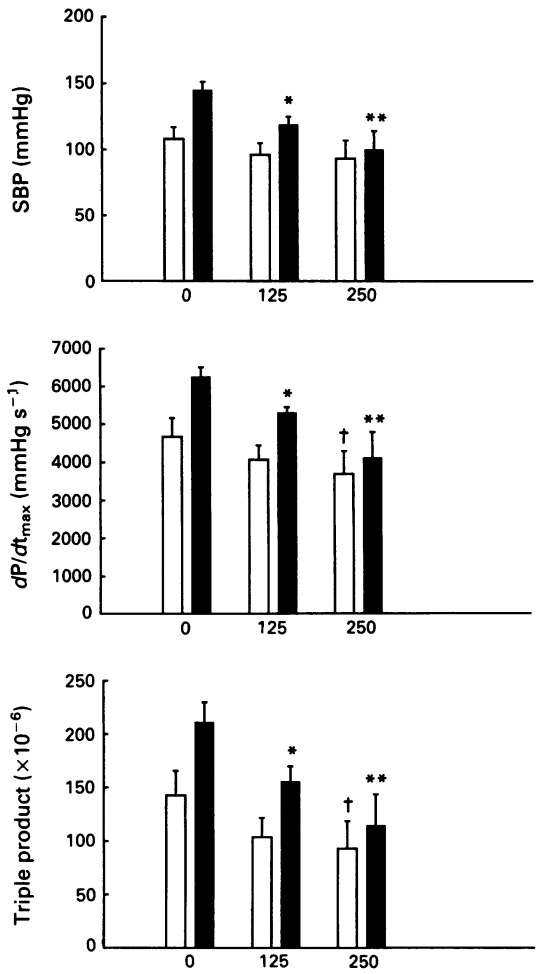

Figure 1 Effects of the intracerebroventricular injection of cumulative doses of 5,7 dichloro-kynurenic acid on cardiovascular responses elicited by electrical stimulation of the paraventricular nucleus of the hypothalamus (PVN) in pentobarbitone anaesthetized rabbits: open columns, control; solid columns, electrical stimulation. SBP: systolic blood pressure. Values are represented as means ± s.e.mean of 5 experiments. $*P<0.05$; $**P<0.01$: significant difference between values observed during stimulation before and after treatment; $\dagger P<0.05$: significant differences between basal values before and after treatment.

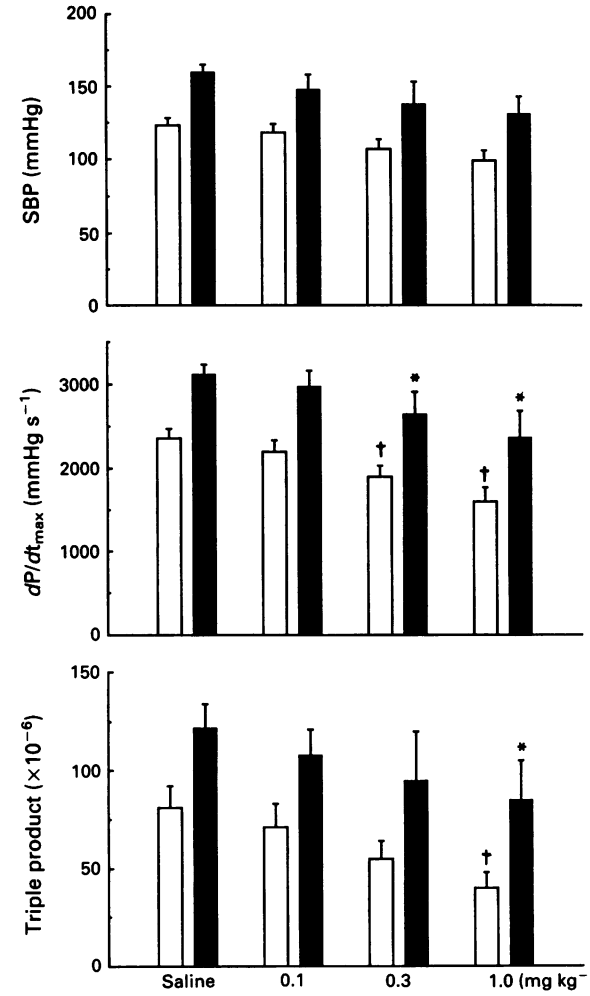

Figure 2 Effects of the intracerebroventricular administration of cumulative doses of 2-amino-5-phosphonovaleric acid (APV) on the cardiovascular responses elicited by electrical stimulation of the paraventricular nucleus of the hypothalamus (PVN) in pentobarbitone anaesthetized rabbits: open column, baseline; solid column, electrical stimulation. Data are represented as means ± s.e.mean of 6 experiments. $*P<0.05$: significant difference between values observed during stimulation before and after treatment; $\dagger P<0.05$: significant differences between basal values before and after treatment.

Table 3 Effects of increasing doses of intracerebroventricular 2-amino-5-phosphonovaleric acid on basal haemodynamics and on the cardiovascular responses to electrical stimulation of the PVN of the pentobarbitone anaesthetized rabbit

	2-Amino-5-phosphonovaleric acid (APV) (mg kg^{-1}, i.c.v.)							
	0		0.1		0.3		1.0	
	C	*S*	*C*	*S*	*C*	*S*	*C*	*S*
dP/dt_{max} (mmHg s^{-1})	2360 ± 111	3120 ± 110	2200 ± 136	2980 ± 179	1900 ± 134†	2650 ± 260*	1600 ± 171†	2370 ± 312*
SBP (mmHg)	123 ± 5	160 ± 5	118 ± 6	148 ± 10	107 ± 6.5	138 ± 11.5	99 ± 7	131 ± 12
DBP (mmHg)	90 ± 5	132 ± 3	87.5 ± 6	122 ± 7	74 ± 5	113 ± 9	73.5 ± 7	108 ± 9
MBP (mmHg)	101 ± 5	141 ± 4	97.5 ± 6	131 ± 8	85 ± 6	123 ± 10	79 ± 6	113 ± 12
HR (beats min^{-1})	266 ± 13	242 ± 13	258 ± 12	236 ± 12	243 ± 13	231 ± 19	230 ± 14	228 ± 15
TP (mmHg^2 s^{-1} b.p.m. 10^{-6})	81 ± 11	122 ± 12	71 ± 12	108 ± 13	55 ± 9	95 ± 25	40 ± 8†	85 ± 20*

C = baseline values; S = electrical stimulation. Each value represents the mean ± s.e.mean of 6 experiments. $*P<0.05$: statistically significant effect of electrical stimulation when compared to values during stimulation before treatment; $\dagger P<0.05$: significant effect of the treatment on basal cardiovascular parameters when compared to control values (ANOVA for repeated measures followed by Scheffé's test). Abbreviations as in Table 1.

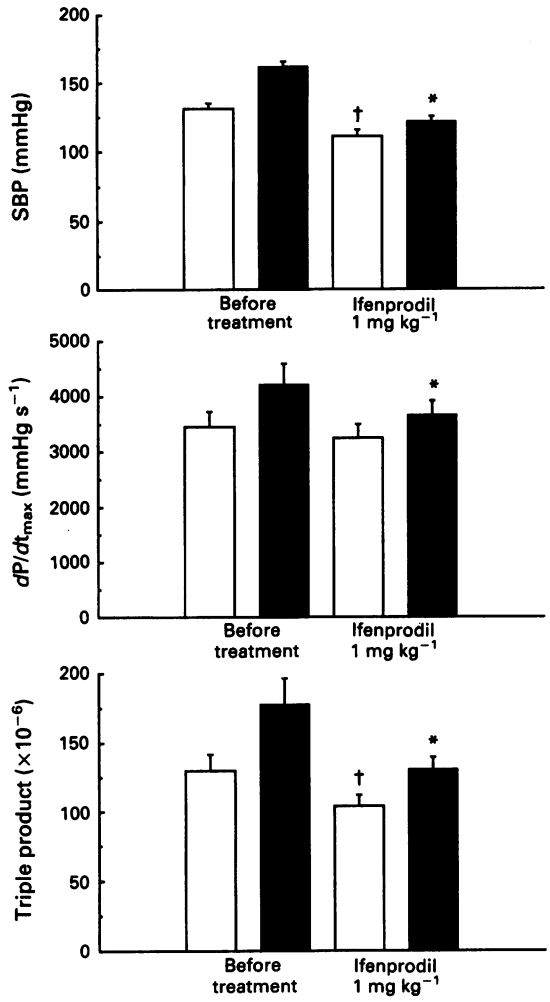

Figure 3 Effects of the intravenous administration of ifenprodil on the cardiovascular responses elicited by electrical stimulation of the paraventricular nucleus of the hypothalamus (PVN) in pentobarbitone anaesthetized rabbits: open columns, baseline; solid columns, stimulation. Data are represented as means ± s.e.mean of 5 experiments. $*P<0.05$: significant difference between values observed during stimulation before and after treatment; $\dagger P<0.05$: significant difference between basal values before and after treatment.

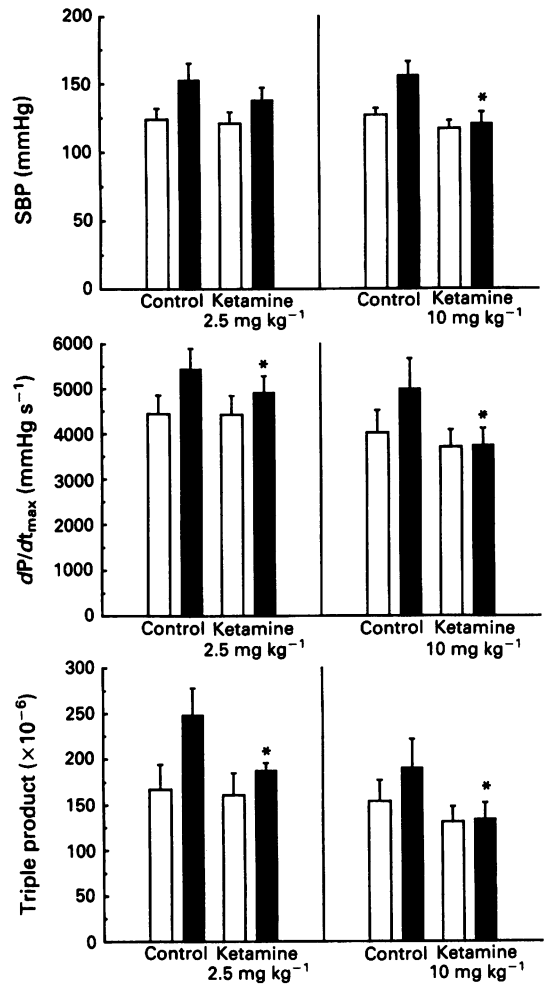

Figure 4 Effects of the intravenous administration of ketamine on the cardiovascular responses elicited by electrical stimulation of the paraventricular nucleus of the hypothalamus (PVN) in pentobarbitone anaesthetized rabbits: open columns, baseline; solid columns, stimulation. Data are represented as means ± s.e.mean of 5 experiments. $*P<0.05$: significant difference between values observed during stimulation before and after treatment.

Table 4 Effects of the intravenous administration of ifenprodil on the cardiovascular response elicited by electrical stimulation of the PVN of the pentobarbitone anaesthetized rabbit

	Before treatment		*Ifenprodil* (1 mg kg^{-1}, i.v.)	
	C	*S*	*C*	*S*
dP/dt_{max} (mmHg s^{-1})	3452 ± 274	4217 ± 373	3250 ± 248	3667 ± 250*
SBP (mmHg)	131 ± 4	162 ± 4	111 ± 5†	122 ± 3*
DBP (mmHg)	104 ± 3	132 ± 3	86 ± 4†	100 ± 3*
MBP (mmHg)	113 ± 3	141 ± 3	95 ± 4†	107 ± 3*
HR (beats min^{-1})	289 ± 16	258 ± 8	292 ± 13	297 ± 15*
TP (mmHg^2 s^{-1} b.p.m. 10^{-6})	130 ± 12	178 ± 18	104 ± 8†	131 ± 9*

C = baseline values; S = electrical stimulation. Each value represents the mean ± s.e.mean of 5 experiments. $*P<0.05$: statistically significant effect of electrical stimulation when compared to values during stimulation before treatment; $\dagger P<0.05$: significant effect of the treatment on basal cardiovascular parameters when compared to control values. (ANOVA for repeated measures followed by Scheffé's test). Abbreviations as in Table 1.

Table 5 Effects of the intravenous administration of ketamine on the cardiovascular response elicited by electrical stimulation of the PVN of the pentobarbitone anaesthetized rabbit

	Ketamine (2.5 mg^{-1}, i.v.)				*Ketamine* (10 mg kg^{-1}, i.v.)			
	Before treatment		*After treatment*		*Before treatment*		*After treatment*	
	C	*S*	*C*	*S*	*C*	*S*	*C*	*S*
dP/dt_{max} (mmHg s^{-1})	4440 ± 415	5447 ± 445	4420 ± 428	4920 ± 361*	4020 ± 486	5000 ± 660	3700 ± 380	3740 ± 370*
SBP (mmHg)	124 ± 8	153 ± 12	121 ± 4	138 ± 8	127 ± 5	156 ± 10	117 ± 6	121 ± 8*
DBP (mmHg)	91 ± 7	123 ± 7	88 ± 7	114 ± 9	91 ± 5	129 ± 8	85 ± 5	96 ± 7*
MBP (mmHg)	102 ± 7	133 ± 9	99 ± 7	122 ± 9	103 ± 4	138 ± 8	96 ± 5	104 ± 7*
HR (beats min^{-1})	281 ± 33	298 ± 12	296 ± 12	286 ± 21	299 ± 8	246 ± 10	298 ± 7	294 ± 7*
TP (mmHg^2 s^{-1} b.p.m. 10^{-6})	155 ± 27	249 ± 29	158 ± 24	188 ± 8*	154 ± 23	191 ± 31	131 ± 17	134 ± 18*

C = baseline values; S = electrical stimulation. Each value represents the mean ± s.e.mean of 5 experiments. *$P<0.05$: statistically significant effect of electrical stimulation when compared to values during stimulation before treatment; (ANOVA for repeated measures followed by Scheffé's test).

Discussion

In the present study, we investigated the possible involvement of a EAA neurotransmitter system in the pathways conducting the excitatory cardiovascular responses elicited by electrical stimulation of the PVN in anaesthetized rabbits.

In untreated animals, the electrical stimulation of the PVN always induced a classical cardiovascular response, i.e. hypertension, bradycardia and increase in dP/dt_{max}. The heart rate response was the most variable, bradycardia being probably due to the baroreflex activation. This excitatory haemodynamic reaction mimicked the one provoked by situations of stress or physical effort in conscious animals (Hilton & Redfern, 1986).

In this model we examined the involvement of NMDA receptors in these responses. Two main receptor subtypes for the EAA are involved in the glutamatergic neurotransmission in the mammalian central nervous system: the N-methyl-D-aspartate receptor/channel complex (NMDA) and the non NMDA receptor, AMPA/kainate (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) (Watkins & Evans, 1981; Collingridge & Lester, 1989; Honore, 1989; Lodge & Johnson, 1990; Watkins *et al.*, 1990). It is well known that the NMDA receptor is widely distributed throughout the brain (Cotman *et al.*, 1987; Sundaram *et al.*, 1989; Young & Fagg, 1990).

5,7 Dichlorokynurenic acid is a derivative of kynurenic acid (a tryptophan metabolite), an endogenous substance that has been shown to block selectively the glutamate-like neurotransmission. It has been demonstrated recently that the EAA antagonist effects of 5,7 diCl-KYNA are due to the selective blocking of the glycine allosteric modulatory unit of the NMDA receptor/channel complex (Johnson & Ascher, 1987; Birch *et al.*, 1988; Foster *et al.*, 1992).

The central administration of 5,7 diCl-KYNA clearly protected the animals against the cardiovascular responses provoked by PVN activation in a dose-related manner with a weak influence on the basal haemodynamic parameters (arterial pressure, heart rate and dP/dt_{max}). This protective effect is unlikely to be due to a diminished neuronal response to electrical stimulation over time (60 min); in control experiments there were no significant differences in the peak values of cardiovascular parameters observed during electrical stimulation of the PVN before and after repetitive i.c.v. administration of saline solution. This was also true with regard to the repetitive intravenous injection of 500 µl of saline solution (data not shown).

Results obtained with 5,7 diCl-KYNA strongly suggested that NMDA receptors were in fact involved in the central effect on the cardiovascular function, in particular the inotropic one.

We also used 2-amino-5-phosphonovaleric acid (APV). APV belongs to the series of the ω-phosphonic-α-carboxylic amino acids developed as antagonists acting on the agonist recognition site of the NMDA receptor (Evans *et al.*, 1982). This acid is a potent and highly selective NMDA antagonist both *in vitro* and in functional studies and is devoid of antagonist effects on AMPA/kainate-induced responses (Davies *et al.*, 1981; Evans *et al.*, 1982; Kehl & McLennan, 1983; Childs *et al.*, 1988). In addition APV exhibited no effects on responses induced by other neurotransmitters or neuromodulators of the central nervous system such as acetylcholine, 5-hydroxytryptamine, noradrenaline or substance P (Childs *et al.*, 1988).

In the present study, APV blunted the excitatory cardiovascular responses evoked by electrical stimulation of the PVN, but it also reduced the resting haemodynamic parameters *per se*, at least at the highest doses used in this study. Therefore, these results suggest that the NMDA recognition agonist site might be involved in the tonic regulation of the cardiovascular function.

In order to characterize this system further, we also used ifenprodil, a NMDA antagonist modulating the polyamine binding site (Carter *et al.*, 1989). This neuroprotective agent has been shown to have cerebral anti-ischaemic properties without producing any behavioural effect (Carter *et al.*, 1988; Gotti *et al.*, 1988). This latter feature of ifenprodil-like NMDA antagonists has made them potential candidates as novel therapeutic agents which could have an application in cardiovascular disorders.

Ifenprodil is a quite interesting drug for at least two reasons: (i) it readily crosses the blood-brain barrier when injected systemically and (ii) it is not a blocker but a modulator of the NMDA receptor/channel complex, and so was expected to block the cardiovascular responses induced by PVN stimulation without inducing any significant cardiodepression. In fact, our results showed that the i.v. injection of ifenprodil reduced the inotropic and vasopressor responses and consequently the increases in the MOD caused by PVN electrical stimulation. The basal arterial blood pressure was also reduced, probably because this drug is also an α-adrenoceptor blocking drug (Chenard *et al.*, 1991). Nevertheless, the classical α-blocker, prazosin, injected intravenously, reduced the vasopressor response but not the increases in dP/dt_{max} induced by electrical stimulation of the PVN (data not shown) while ifenprodil also affected the inotropic response.

Numerous findings suggest that the 'dissociative anaes-

thetics' like ketamine (which is a lipophilic compound and readily crosses the blood-brain barrier) are non-competitive antagonists of the EAA neurotransmission system (Martin & Lodge, 1985; Collingridge & Lester, 1989; Lodge & Johnson, 1990). It has been shown that ketamine binds to a specific site within the NMDA ion channel complex, called the phencyclidine site (PCP site) (Anis *et al.*, 1983; Honey *et al.*, 1985; Davies *et al.*, 1988; Halliwell *et al.*, 1989; MacDonald & Novak, 1990).

We wished to determine whether the influence of the blockade of glutamate effects induced by competitive and non-competitive NMDA antagonists on nervous regulation would be different. In fact, if the cardiovascular responses provoked by electrical stimulation of the PVN were due to the conversion of the cation channel coupled to the NMDA receptor from the closed to the open state, it is tempting to speculate that ketamine would inhibit the response without affecting the resting cardiovascular parameters. Indeed, in our experimental model, the i.v. injection of ketamine (2.5 and 10 mg kg^{-1}) prevented not only the hypertensive and positive inotropic responses to electrical stimulation of the PVN but also the increase of the triple product. These data showed that this drug is capable of inducing a central cardioprotective effect. It is noteworthy that these doses of ketamine are quite low, i.e. about 10 times lower than the anaesthetic ones in this species. Interestingly, the protective effects of ketamine, which were related to the dose, did not significantly reduce the resting haemodynamic parameters.

Thus, the results obtained with these two latter drugs injected via the i.v. route, are very similar to those observed with 5,7 diCl-KYNA and APV. The common effect of all these drugs was their blockade of the central NMDA receptors, suggesting that they blunted the cardiovascular responses to PVN stimulation probably by inhibiting the link(s) in the central nervous command to the cardiac function, including cardiac contractility, operating by EAA as neurotransmitters.

As a consequence of their inhibitory effect on the haemodynamic responses to hypothalamic stimulation, all the EAA inhibitors decreased oxygen consumption as measured by the triple product index. We recently described a similar action of baclofen (Tibiriça *et al.*, 1993), a $GABA_B$ agonist, known to inhibit the presynaptic release of glutamate (Bowery *et al.*, 1980).

Thus, modulation of the central regulation of cardiac function with drugs acting on glutamatergic structures, might open a new therapeutic perspective i.e. non-psychotropic centrally-acting cardioprotective drugs. Whether or not some specific antagonists of the NMDA receptor sites will be prototypes has to be investigated further. However, the data we obtained here, especially with ifenprodil, known to be devoid of any psychotropic toxicity, already look promising.

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