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Inhibition of the centrally induced increases in myocardial oxygen demand in rabbits by chronic treatment with baclofen, a selective $GABA_B$ agonist

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1 A previous study from our group demonstrated that neurones of the paraventricular nucleus of the hypothalamus (PVN) are selectively involved in the central control of the cardiac function. Moreover, in that study, it was shown that baclofen, a selective $GABA_B$ receptor agonist, is capable of modulating the increases in myocardial contractility and oxygen demand evoked by electrical or pharmacological stimulation of the PVN. Nevertheless, the acute administration of this compound was frequently accompanied by a cardiodepressant effect.

2 In the present study, the effects of a long term treatment (14 days) with baclofen (3 or 10 mg kg^{-1}, i.p.) have been examined on the excitatory haemodynamic responses evoked by central pharmacological stimulation in anaesthetized rabbits.

3 The i.c.v. injection of L-glutamate (3 mg kg^{-1}) induced marked increases in dP/dt_{max} (32%), mean arterial pressure (39%) and on two indices of myocardial oxygen consumption: the rate-pressure product (34%) and the triple product (78%).

4 Baclofen blunted the positive inotropic response and the increases in myocardial oxygen consumption induced by L-glutamate in a dose-related manner. The higher dose of baclofen (10 mg kg^{-1}, i.p.), reduced by more than 50% these excitatory effects of L-glutamate without eliciting any significant negative effect on basal haemodynamics. The same doses of baclofen were not able to blunt the hypertensive response induced by central stimulation.

5 These results confirm and extend our previous findings suggesting that it is possible to discriminate the central control of vasomotor tone from that of cardiac function and also that baclofen can modulate the latter. It is concluded that when given chronically, baclofen modulates the increases in myocardial oxygen demand induced by activation of the central nervous system in doses which do not depress the resting cardiac function.

Keywords: Baclofen; myocardial contractility; myocardial oxygen consumption; $GABA_B$ receptors; L-glutamate; cardioprotection

Introduction

In a previous study (Tibiriçà *et al.*, 1993), it was demonstrated that an inhibitory GABAergic system of neurotransmission is involved in the central modulation of the cardiac inotropic drive. In fact, the activation of GABA receptors by the central or systemic administration of baclofen, a selective $GABA_B$ receptor agonist (Bowery *et al.*, 1979), blunts the marked increases of the cardiac contractility indices and myocardial oxygen demand induced by hypothalamic stimulation in rabbits (Tibiriçà *et al.*, 1993). The inhibitory effect of baclofen could be explained by its well-known ability to decrease the presynaptic release of excitatory neurotransmitters like glutamate, which is mediated by hyperpolarization of the neurones via increased K^+ conductance (Bowery *et al.*, 1980; Lanthorn & Cotman, 1981; Raiteri *et al.*, 1989; Otero-Losada & Acosta, 1992). Indeed, in a recent study we showed that the pretreatment of the animals with four different glutamate antagonists acting on different binding sites of the NMDA receptor/channel complex also dose-dependently blunts the excitatory haemodynamic effects elicited by central stimulation (Monassier *et al.*, 1994). Thus, we suggested that drugs modulating the activity of the GABAergic and/or glutamatergic systems of neurotransmission could be useful centrally acting cardioprotective drugs (Tibiriçà *et al.*, 1993; Monassier *et al.*, 1994).

Nevertheless, the cardioprotective effects of baclofen observed in our previous experiments were accompanied by a reduction of basal haemodynamics, at least following acute (single) intracerebroventricular administration (0.1-1 $\mu g\ kg^{-1}$) (Tibiriçà *et al.*, 1993). The cardiovascular inhibitory effects of baclofen could be accounted for by the administration of rather high bolus doses, which were necessary to achieve active concentrations in the brain rapidly. However, the progressive elevation of the plasma levels of baclofen and slow penetration of the drug into the central nervous system (Faigle & Keberle, 1972) that are likely to be obtained with long term treatment, could be devoid of cardiodepressant effects. In view of these possibilities, the present study was designed to determine the effects of chronic treatment of rabbits with baclofen on the basal haemodynamics and on the cardiovascular excitatory effects of central nervous system stimulation.

Thus, we developed an experimental model reproducing the haemodynamic effects of stressful situations and physical effort. The i.c.v. injection of L-glutamate in the anaesthetized rabbit, as was the case for electrical or pharmacological stimulation of the hypothalamus (Tibiriçà *et al.*, 1993), also induces marked increases in the cardiac contractility and myocardial oxygen demand indices.

In the present paper, we describe the cardioprotective effects of long term treatment with baclofen in this experimental model.

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Methods

Experimental protocol

During the 14 days before the surgical procedure, the animals received twice daily intraperitoneal injections of saline (control group, $n=9$) or intraperitoneal administration of baclofen [3 mg kg^{-1} total dose per day ($n=7$) or 10 mg kg^{-1} ($n=9$)] in a constant volume of 1 ml kg^{-1}.

Animals and treatments were assigned in a random fashion. On the day of the final experiment, which always took place at 08 h 00 min, the animals received the last dose of baclofen and were anaesthetized and prepared as described below. The weight of the animals was measured on days 0, 7 and 14, in order to detect some influence of the treatment on the general state of the animals. In addition, neither inhibition of the locomotor activity nor sedation were observed in the baclofen-treated animals. The haemodynamic responses reported throughout this paper were recorded at the moment of the peak effect elicited by central glutamate administration.

Animals and haemodynamic measurements

New Zealand albino rabbits of either sex weighing from 2 to 3 kg (from the Oswaldo Cruz Foundation breeding facilities) were anaesthetized with sodium pentobarbitone (40 mg kg^{-1}) administered through the marginal vein of the ear; anaesthesia was complemented by another i.v. injection of 5 mg kg^{-1} of pentobarbitone before the control period and when necessary (see below). The rabbits were intubated with a polyethylene tube via a tracheotomy and immobilized with pancuronium bromide (1 mg kg^{-1}, i.v.) with hourly supplemental doses of 0.2 mg kg^{-1} and artificially ventilated with room air (tidal volume 10 ml kg^{-1}; stroke rate 25 min^{-1}). The right femoral vein was catheterized to permit i.v. injections. The arterial pressure was continuously monitored through a catheter placed in the abdominal aorta via the right femoral artery which was connected to a Hewlett Packard quartz transducer (1290 A), which in turn was connected to a pressure processor and recorder (Hewlett Packard 7754 system with 8805B amplifier). Systolic (SAP) and diastolic (DAP) arterial pressures were obtained directly from the recordings and mean arterial pressure (MAP) was calculated as diastolic pressure plus one-third of the differential pressure; heart rate (HR) was counted from the blood pressure waves by rapid running of the pressure recording. After completion of the surgical procedures the animals were allowed to equilibrate for 15–30 min or until a stable tracing had been obtained (control period). Before injection of drugs, the means of 3 values of arterial pressures (SAP, DAP and MAP) and HR recorded at 5 min intervals were calculated and considered as the basal haemodynamic values. Left ventricular pressures and the maximum rate of rise of left ventricular pressure (dP/dt_{max}) were measured with a high fidelity micromanometer-tipped catheter (Gaeltec Ltd, model ICT/B, Dunvegan Scotland) 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 'rate-pressure product' (RPP) was calculated by multiplying the heart rate by the systolic blood pressure and dividing the product by 1000 to reduce it to convenient units. The 'triple product' designates the RPP multiplied by the dP/dt_{max} divided by 10^6. This product will be referred to subsequently as the 'triple product'. The RPP and the triple product will be expressed throughout this article in mmHg b.p.m.$^{-1}$ 10^{-3} and $mmHg^2$ s^{-1} b.p.m.$^{-1}$ 10^{-6}, respectively.

Intracerebral injections

The head of the animal was fixed in a stereotaxic apparatus (Unimécanique, Epinay/Seine, France). A craniotomy was performed and the dura mater cut, a 26-gauge needle connected to a Hamilton microlitre syringe (Hamilton Bonaduz AG, Switzerland) was inserted in the following stereotaxic coordinates: AP: −4.5 mm from bregma; L: −8.0 and −6.0 mm down from the cranial surface (Sawyer *et al.*, 1954) to permit stereotaxic drug injections in the left lateral ventricle. Another cannula (26-gauge stainless-steel hypodermic needle) was placed in the cisterna magna through the atlanto-occipital membrane in order to permit the free circulation of the cerebrospinal fluid and drug solutions to avoid intracranial hypertension. Normal saline solution (control group) or L-glutamate were injected in a constant volume of 100 µl of saline solution. At the end of each experiment, the same volume of Evans Blue dye was injected under the same conditions. The brain was then removed *post-mortem* and dissected to check that the drugs had diffused properly throughout the ventricular space.

Drugs

The following drugs were used: sodium pentobarbitone (Nembutal, Abbott lab., North Chicago, IL, U.S.A.); pancuronium bromide (Pavulon, Organon Teknika, Fresnes, France); baclofen and L-glutamate (Sigma Chemical Co, St Louis, MO, U.S.A.). For both the i.p. or i.c.v. injections the drugs were dissolved in normal saline solution (0.9% NaCl).

Statistical analysis

All results are expressed as means ± s.e.mean for n experiments. The haemodynamic responses to glutamate injections were analysed by Student's paired t test. Comparisons between the haemodynamic parameters before and after glutamate injection on the control group (saline-injected) and treated groups (animals injected with baclofen in saline solution) were made with one-way ANOVA followed by the Scheffé's test (Wallenstein *et al.*, 1980) to localize the statistically significant differences. All calculations were made by computer-assisted analyses using a commercially available statistical package (Primer Program, McGraw-Hill Inc. Version 1.0; 1988).

Results

Control experiments and basal values

There were no significant differences in the mean basal values of the dP/dt_{max}, RPP, triple product and diastolic pressure measured in this study between the different experimental groups.

The i.p. treatment of the animals during 14 days with normal saline solution (1 ml kg^{-1}) did not change the cardiovascular response to central injection of glutamate (3 mg kg^{-1}), when compared to naive animals (data not shown). Moreover, there were no significant differences in the variations of the body weight of the animals between the different experimental groups, as measured at the end of the treatment period.

Haemodynamic effects of the i.c.v. injection of glutamate

The i.c.v. administration of glutamate (3 mg kg^{-1}) in pentobarbitone-anaesthetized rabbits induced significant increases in dP/dt_{max} that reached a maximum of 32% above baseline values ($n=9$; $P<0.05$) (Table 1). The positive inotropic response was accompanied by rises in mean arterial pressure, reaching a peak value of 39%, when compared to the initial value of 86 mmHg ($n=9$; $P<0.05$) (Table 1). We also observed a mild increase in heart rate, which was not statistically significant (262 b.p.m. before and 271 b.p.m. after glutamate injection; $n=9$; $P>0.05$). These positive inotropic and hypertensive responses led to marked increases in the indices of myocardial oxygen demand: the RPP increased by 34% ($n=9$; $P<0.05$) and the triple product by 78% ($n=9$; $P<0.05$) (Figure 1).

Table 1 Effects of a chronic treatment with baclofen (14 days) on the excitatory haemodynamic responses evoked by glutamate (3 mg kg^{-1}, i.c.v.) on anaesthetized rabbits

	Saline ($n=9$)			*Baclofen* (mg kg^{-1}, i.p.) *3* ($n=7$)			*10* ($n=9$)		
	Basal	*Glu*	Δ%	*Basal*	*Glu*	Δ%	*Basal*	*Glu*	Δ%
dP/dt_{max} (mmHg s^{-1})	4166±174	5477±201.3	32.3±4.6	4585±213.9	5671±282.5	23.9±4.1	4400±265.5	5011±338.5	14.4±5.5
SAP (mmHg)	113±5	145±4.3	28.6±4.4	120±2.6	144±6	20±3.9	118±1.6	147±2.8	24±2.9
DAP (mmHg)	73±4.1	107±5.1	47.6±3.5	74±2.7	112±8.5	50.3±8.1	75±1.9	108±5.2	44±5.2
MAP (mmHg)	86±4.2	120±4.7	39.3±3.8	90±2.3	121±8	34.3±6.9	89±1.4	121±3.8	35.3±3.6
HR (b.p.m.)	262±9.6	271±10.6	5.2±1.9	263±8.7	266±13	0.8±2.1	267±7.1	244±12.1	−8.3*±3.7
RPP (mmHg b.p.m.$^{-1}\times10^{-3}$)	29.9±2.1	39.8±2.5	33.7±3.1	31.5±1.4	38.4±2.8	21.2*±5.5	31.5±0.9	35.8±1.9	13.7*±5.6
TP (mmHg^2 b.p.m.$^{-1}$ s$^{-1}\times10^{-6}$)	126.7±13.5	220.3±20.3	77.7±9.5	145±10.7	217.8±18	50.6±9.4	139±9.8	180.5±17.6	31.2*±11.2

Basal = baseline values; Glu = maximum values after glutamate administration; dP/dt_{max}, maximum rate of rise of left ventricular pressure; MAP = mean arterial pressure; SAP = systolic arterial pressure; DAP = diastolic arterial pressure; HR = heart rate; RPP = (rate-pressure product) SAP × HR; TP (triple product) SAPxHRxdP/dt_{max}.
n = number of experiments
*$P<0.05$ significant effect of the treatment on haemodynamic responses to central glutamate administration.

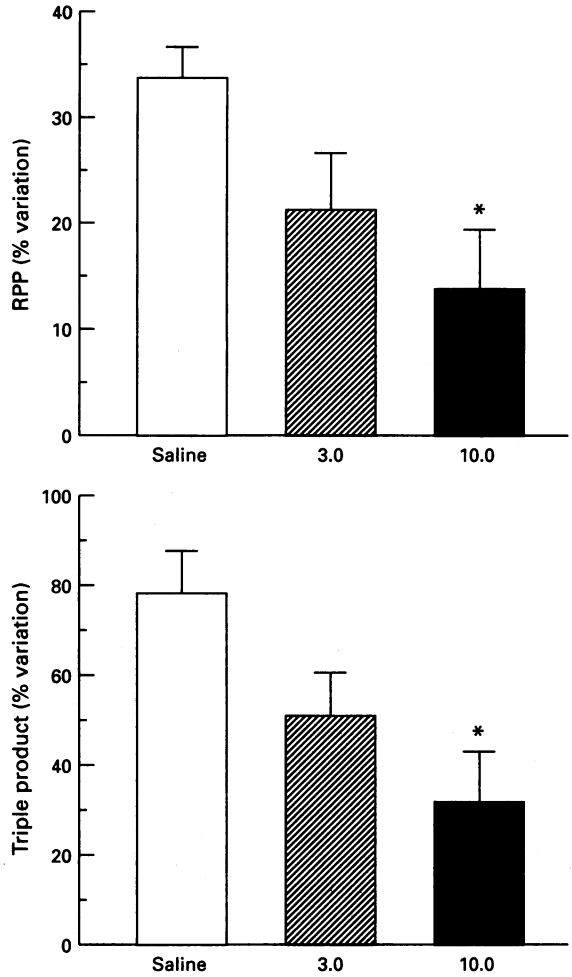

Figure 1 Mean percentage variations of the myocardial oxygen demand indices by i.c.v. administration of L-glutamate (3 mg kg^{-1}) in saline-injected or baclofen-treated (14 days) anaesthetized rabbits.

Effects of chronic treatment with baclofen on the haemodynamic response to i.c.v. glutamate administration

Two groups of animals were treated for 14 days with i.p. injections of baclofen (1.5 or 5 mg kg^{-1} twice a day). The increase in dP/dt_{max} evoked by i.c.v. glutamate was attenuated by baclofen; in the group treated with the highest dose (10 mg kg^{-1} day^{-1}) the dP/dt_{max} increased by only 14% ($n=9$; $P<0.05$) (Table 1), when compared to the animals treated with saline solution (32%). Even at the highest dose, the increases of systolic, diastolic and mean arterial pressures induced by glutamate were not significantly affected by baclofen. As a consequence, the increases of RPP and of TP of 34% and of 78% observed in the saline-treated group were only of 14% and of 31%, respectively, in the 10 mg kg^{-1} day^{-1} group (Table 1). At this dose, the heart rate response to glutamate injection was clearly a bradycardia (−8±4%) after baclofen administration. Whereas it was a weak tachycardia in the absence of the drug.

Discussion

We recently showed that the electrical stimulation of the paraventricular nucleus of the hypothalamus (PVN) elicits positive inotropic and pressor effects which are accompanied by marked increases in myocardial oxygen demand indices that appear to be mediated by an increase in the sympathetic outflow.

Furthermore, the selective pharmacological activation of neuronal cell bodies localized in the PVN with glutamic acid and kainic acid evokes positive inotropic effects with few effects on arterial pressure. These latter findings led us to suggest the existence of a hypothalamic neuronal population able to modulate the cardiac inotropic drive that could be, at least in part, independent from the neural pathways that regulate arterial pressure (Tibiriçà *et al.*, 1993).

We also demonstrated that the central ($0.1-1$ $\mu g\ kg^{-1}$) or systemic ($1-10$ $mg\ kg^{-1}$) acute administrations of the selective $GABA_B$ receptor agonist, baclofen, or of several antagonists of the NMDA receptor/channel complex blunt the excitatory haemodynamic responses elicited by central nervous system (CNS) stimulation (Monassier *et al.*, 1994). This protective effect of baclofen cannot be attributed to a direct action on the heart. In fact, the rather low doses of the drug (0.1 to 1.0 $\mu g\ kg^{-1}$) that were active upon intracerebroventricular administration, proved to be totally inactive when injected by the intravenous route (Tibiriçà *et al.*, 1993).

Baclofen is a lipophilic derivative of γ-aminobutyric acid (GABA) that crosses the blood-brain barrier and is used in the treatment of spasticity syndromes resulting from lesions of the CNS (Bowery *et al.*, 1980; Bowery, 1982) and whose main site of action appears to be the spinal cord (Fukuda *et al.*, 1977). Spinal glutamatergic relays on the sympathetic pathways regulating cardiovascular function have been described (Morrison *et al.*, 1989; Chalmers & Pilowsky, 1991). It has already been demonstrated that baclofen selectively inhibits the release of excitatory amino acids like aspartate and glutamate (Johnston *et al.*, 1980; Davies, 1981; Lanthorn & Cotman, 1981). In fact, the activation of $GABA_B$ receptors, which are also located on nerve terminals, leads to the inhibition of neurotransmitter release via the opening of K^+ channels and consequent neuronal hyperpolarization (Bowery *et al.*, 1980; Newberry & Nicoll, 1984; 1985; Raiteri *et al.*, 1989; Potier & Dutar, 1993) or inhibition of calcium currents (Zhu & Chuang, 1987; Allerton *et al.*, 1989; Maguire *et al.*, 1989). Furthermore, the neuronal inhibitory effects of low doses of systemically administered baclofen, which correspond to the dose-range used in this study, on the synaptic excitatory transmission in the CNS, is mediated by a presynaptic action (Pierau & Zimmerman, 1973; Fox *et al.*, 1978; Davies, 1981). Concerning the centrally-mediated cardioprotective effects of baclofen, one hypothesis could be that the drug inhibits the presynaptic neurotransmitter release of the sympathetic pathways.

The present study provides further evidence for a modulatory effect of baclofen on the central control of the cardiac inotropic drive.

Our results showed that the i.c.v. administration of L-glutamic acid ($3\ mg\ kg^{-1}$) increases dP/dt_{max}, indices of myocardial oxygen consumption and blood pressure. The heart rate is the single cardiovascular parameter that was not significantly increased; the activation of the baroreceptor reflex elicited by the marked rises in arterial pressure may have masked the potential tachycardic effect of central glutamate administration. These effects were of the same magnitude as those observed upon electrical or pharmacological stimulation of the PVN (Tibiriçà *et al.*, 1993; Monassier *et al.*, 1994). Indeed, the sharp rises of the triple product, which is closely related to directly measured myocardial oxygen consumption (Baller *et al.*, 1979), reached the maximum of more than 70% above baseline levels. It is obvious that these sudden and marked increases in the metabolic needs of the heart can elicit deleterious effects on the ischaemic myocardium. Thus, we hypothesized that the modulation of the cardiovascular responses elicited by the activation of the CNS commonly observed in daily life, such as stressful situations and physical effort (Lown *et al.*, 1977; Reich *et al.*, 1981; Specchia *et al.*, 1984), would be achieved with pharmacological agents devoid of cardiodepressive effects. Such drugs would constitute a new family of therapeutic agents, namely central cardioprotective agents.

Our results also showed that the chronic treatment of the animals with baclofen dose-dependently reduced the excitatory cardiovascular response evoked by pharmacological stimulation of the CNS. Accordingly, there was an impressive reduction of the myocardial oxygen demand, as measured by the rate-pressure and triple products. It is noteworthy that there is a close relationship between the levels of rate-pressure product reached during physical effort and/or emotional stress and the outbreak of ischaemic episodes in patients with coronary artery disease (Robinson, 1967; Specchia *et al.*, 1984). Moreover, the triple product appears to be even more reliable than the RPP in the evaluation of the myocardial oxygen demand as it takes into account the changes in cardiac contractility, which is a determinant of the cardiac work.

In the present work, the cardiac contractility was only evaluated by the maximum rate of rise of left ventricular pressure (dP/dt_{max}). In previous studies, we observed that in our experimental models this cardiovascular parameter was very similar to calculated indices of myocardial contractility, which take into account the instantaneous or the developed pressures in the left ventricle, and is to some extent, independent of the loading conditions imposed on the heart (Tibiriçà *et al.*, 1993).

Interestingly, the doses of baclofen that showed the ability to induce cardioprotection in our experimental model, did not evoke any basal haemodynamic inhibitory effect. In fact, the levels of the cardiovascular parameters of the animals treated with increasing doses of baclofen were not different from those of the animals that received i.p. injections of the vehicle. Furthermore, we demonstrated in a separate group of animals, that in the 2 h following the i.p. injection of baclofen no reduction of the cardiovascular parameters could be detected.

We also observed that the treatment of the animals with baclofen during two weeks afforded a selective protection against the acute increases in the dP/dt_{max}. In addition at least the highest dose of baclofen converted the weak glutamate-induced tachycardia into bradycardia. As a result, baclofen significantly reduced the myocardial oxygen demand elicted by CNS stimulation. While the increases in dP/dt_{max} and myocardial oxygen demand indices were reduced by more than 50% by baclofen, the hypertensive effect was not affected at all by the treatment. These latter results support our hypothesis according to which the neural pathways involved in the central regulation of arterial pressure can be dissociated from the ones regulating cardiac function (Tibiriçà *et al.*, 1993).

In summary, our data show that chronic treatment with baclofen inhibits the centrally-evoked increases in cardiac contractility and myocardial oxygen demand without inducing any depression of the basal haemodynamic parameters.

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