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$GABA_B$ receptor-mediated inhibition of the neurogenic vasopressor response in the pithed rat[1]

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1 The effects of γ-aminobutyric acid (GABA) and related drugs on the vasopressor response induced by electrical stimulation (single pulse of 30 V and 1 ms) of the preganglionic sympathetic nerve fibres or by injection of noradrenaline 0.3 nmol kg^{-1} were studied in the pithed rat.

2 The electrically-induced increase in diastolic blood pressure was inhibited by GABA and the $GABA_B$-receptor agonist **R**-(−)-baclofen but was not affected by its **S**-(+)-enantiomer and by the $GABA_A$-receptor agonists muscimol and 3-aminopropane sulphonic acid.

3 The dose-response curve of **R**-(−)-baclofen for its inhibitory effect on the electrically-induced vasopressor response was shifted to the right by the $GABA_B$-receptor antagonist 2-hydroxysaclofen, but was not affected by the $GABA_A$-receptor antagonist bicuculline. 2-Hydroxysaclofen and bicuculline by themselves did not affect the electrically-induced vasopressor response.

4 The increase in diastolic blood pressure induced by exogenous noradrenaline was not affected by the GABA-related drugs, which also had no (or very slight) effects on the basal diastolic blood pressure.

5 It is concluded that GABA inhibits catecholamine release in the resistance vessels of the rat via $GABA_B$-receptors, probably located presynaptically on the postganglionic sympathetic nerve fibres.

Introduction

γ-Aminobutyric acid (GABA) is involved in the central regulation of cardiovascular functions. Thus, intracerebral or intracisternal administration of GABA, GABA receptor agonists and antagonists produces alterations of blood pressure and heart rate (for review, see DeFeudis, 1983; Antonaccio, 1984; Bousquet *et al.*, 1985). In addition, GABA is also capable of relaxing vascular smooth muscle by activation of postsynaptic $GABA_A$- receptors located on the vessels themselves, and locally formed GABA might play a role *in vivo* in the cerebral vascular bed at least under pathophysiological conditions (for review, see Krause, 1986). In some isolated vessels of the rabbit (Starke & Weitzell, 1980; Anwar & Mason, 1982; Manzini *et al.*, 1985) and in middle cerebral arteries of the goat (Miranda *et al.*, 1989), presynaptic $GABA_B$-receptors could be identified, activation of which produces an inhibition of the stimulation-evoked noradrenaline release and/or contraction. Miranda *et al.* (1989) could also show that, in the anaesthetized goat, the decrease in cerebral blood flow induced by electrical stimulation of the cervical sympathetic nerves was diminished by GABA and baclofen via $GABA_B$-receptors.

The question of whether presynaptic $GABA_B$-receptors are also present in vessels of the rat and, more importantly, whether peripherally administered GABA might influence blood pressure via $GABA_B$-receptors in the resistance vessels has not been addressed. Therefore, we examined the effects of GABA and related drugs on the neurogenic vasopressor response in the pithed rat.

Methods

Male Wistar rats weighing 170–420 g were anaesthetized with methohexitone 300 μmol kg^{-1} i.p. and then injected i.p. with atropine 2 μmol kg^{-1}. Following cannulation of the trachea, the animals were pithed and artificially respired with air (60 strokes min^{-1}). Both vagi were cut. Arterial blood pressure was measured from the right carotid artery via a Statham P 23 ID pressure transducer (Statham Instruments, Puerto Rico) and was recorded on a Hellige Servomed (Hellige, Freiburg, F.R.G.). The left jugular vein was cannulated for i.v. injections of drugs. Body temperature was kept constant via a thermostatically controlled heating table.

Subsequent to i.v. injection of (+)-tubocurarine 1.3 μmol kg^{-1}, an increase in blood pressure was induced four times (S_1, S_2, S_3, S_4) either by an i.v. injection of noradrenaline 0.3 nmol kg^{-1} or by a single electrical pulse (30 V, 1 ms; delivered from a Stimulator T; Hugo Sachs, March-Hugstetten, F.R.G.) generated between the pithing rod and an indifferent electrode placed dorsally (according to Gillespie & Muir, 1967). GABA-receptor antagonists, their vehicle (water) or saline (other experiments) were administered 5 min after injection of (+)-tubocurarine and 5 min before S_1. GABA-receptor agonists (or their vehicles) were injected i.v. in increasing doses 5 min before S_2, S_3 and S_4. An interval of 7 min elapsed between two subsequent injections of noradrenaline or electrical stimuli.

In the series described in the last paragraph of Results, 30 s periods of electrical stimulation (2 Hz, 30 V, 1 ms) instead of single electrical pulses were administered.

Calculations and statistics

Results are given as means ± s.e.mean throughout the paper (n: number of rats). In order to quantify the effects of drugs on the rise in diastolic blood pressure induced electrically or by injection of noradrenaline, the ratios S_2/S_1, S_3/S_1 and S_4/S_1 were determined. For quantification of drug-induced effects on the basal blood pressure the ratio of the basal blood pressure immediately before S_2 (t_2), S_3 (t_3) or S_4 (t_4) over that immediately before S_1 (t_1) was determined (t_2/t_1, t_3/t_1 and t_4/t_1). Ratios were expressed as percentages of the corresponding ratios obtained from animals which received the vehicle instead of the drug. For statistical comparison of the corresponding S_n/S_1 and t_n/t_1 values from drug- and vehicle-treated animals, Student's t test was used. If two or more experimental series were compared to the same control series, the t test was subjected to Bonferroni's procedure.

Drugs used

3-Aminopropane sulphonic acid (sodium salt), γ-aminobutyric acid (GABA), atropine sulphate, muscimol, (−)-noradrenaline bitartrate, (+)-tubocurarine chloride (Sigma, Munich, F.R.G.); **R**-(−)-, **S**-(+)-baclofen hydro-

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Table 1 Effects of bicuculline and 2-hydroxysaclofen on basal diastolic blood pressure and on the electrically- and noradrenaline-induced rise in diastolic blood pressure (BP) in pithed rats

	Control	*Bicuculline* ($10\,\mu mol\,kg^{-1}$)	*2-Hydroxysaclofen* ($50\,\mu mol\,kg^{-1}$)
Basal BP (mmHg)	55.2 ± 1.5	51.3 ± 1.4	49.0 ± 1.0*
Rise in BP (mmHg) induced by exogenous noradrenaline	18.5 ± 1.4	17.8 ± 1.9	16.7 ± 1.8
Electrically-induced rise in BP (mmHg)	15.3 ± 1.1	13.1 ± 1.1	15.7 ± 1.3

Pithed rats received an i.v. injection of bicuculline, 2-hydroxysaclofen or water (vehicle for the antagonists; control). Five min later, noradrenaline $0.3\,nmol\,kg^{-1}$ was injected i.v. or a single electrical pulse of 30 V and 1 ms was administered. Basal blood pressure was determined immediately before the injection of noradrenaline or the administration of the electrical stimulus. Data shown are means ± s.e.mean of 8–18 experiments.
* $P < 0.01$ compared to the control.

chloride (CIBA-Geigy, Basle, Switzerland); (+)-bicuculline methiodide (Bioscience Products, Emmenbrücke, Switzerland or Sigma, Munich, F.R.G.; according to Simonyi *et al.* (1989), the correct chemical name of this drug is (−)-bicuculline methiodide); 2-hydroxysaclofen (Tocris Neuramin, Buckhurst Hill, U.K.); methohexitone sodium (Brevimytal Natrium; Lilly, Giessen, F.R.G.).

Stock solutions of most of the drugs were prepared in saline and diluted with saline to the concentration required. Noradrenaline was dissolved and diluted in saline containing ascorbic acid $6\,mmol\,l^{-1}$. Methohexitone, bicuculline and 2-hydroxysaclofen were dissolved in water; muscimol was dissolved in HCl $0.05\,mol\,l^{-1}$. Drugs were injected in a volume of $0.5\,ml\,kg^{-1}$, $1\,ml\,kg^{-1}$ (atropine, (+)-tubocurarine),

Table 2 Effect of R-(−)-baclofen on basal diastolic blood pressure and on the electrically- and noradrenaline-induced rise in diastolic blood pressure (BP) in pithed rats

Parameter studied	*Experimental conditions*	t_1 or S_1 (mmHg)	t_2/t_1 or S_2/S_1	t_3/t_1 or S_3/S_1	t_4/t_1 or S_4/S_1
Basal BP	Control	48.1 ± 1.3	1.04 ± 0.01	1.02 ± 0.02	1.00 ± 0.03
	R-(−)-Baclofen	49.3 ± 1.5	1.00 ± 0.01	1.02 ± 0.02	1.03 ± 0.03
Rise in BP induced by exogenous noradrenaline	Control	16.5 ± 1.5	1.15 ± 0.10	1.14 ± 0.06	1.10 ± 0.17
	R-(−)-Baclofen	15.0 ± 1.8	1.03 ± 0.06	1.01 ± 0.10	0.97 ± 0.10
Electrically-induced rise in BP	Control	15.8 ± 1.8	1.05 ± 0.05	1.08 ± 0.05	1.10 ± 0.05
	R-(−)-Baclofen	17.7 ± 2.1	0.77 ± 0.03*	0.54 ± 0.03**	0.53 ± 0.04**

Four stimuli (S_1–S_4; i.v. injections of noradrenaline $0.3\,nmol\,kg^{-1}$ or single electrical pulses of 30 V and 1 ms) were administered to pithed rats at intervals of 7 min. R-(−)-Baclofen was injected i.v. in three increasing doses 5 min before S_2 ($1\,\mu mol\,kg^{-1}$), S_3 ($10\,\mu mol\,kg^{-1}$) and S_4 ($100\,\mu mol\,kg^{-1}$), whereas saline was injected 5 min before S_1. In the control series, saline was given 5 min before S_1–S_4. The ratios of the rise in blood pressure evoked by S_2, S_3 or S_4 over that evoked by S_1 were determined. To quantify the effects of R-(−)-baclofen on basal blood pressure, the ratios of the blood pressure immediately before S_2, S_3 or S_4 (t_2, t_3 and t_4) over that before S_1 (t_1) were calculated (t_2/t_1, t_3/t_1 and t_4/t_1). Data shown are means ± s.e.mean of 5–12 experiments.
*$P < 0.005$, **$P < 0.001$ compared to the corresponding control.

Table 3 Effects of GABA receptor agonists on basal diastolic blood pressure and on the noradrenaline-induced rise in diastolic blood pressure (BP) in pithed rats

Parameter studied	*Agonist*	*Dose of agonist* ($\mu mol\,kg^{-1}$) *0.1*	*1*	*10*	*100*	*300*
Basal BP	GABA	—	—	94 ± 1*	93 ± 2	98 ± 2
	R-(−)-Baclofen	90 ± 2*	96 ± 1	100 ± 2	103 ± 3	—
	S-(+)-Baclofen	—	—	99 ± 1	107 ± 3	109 ± 2
	Muscimol	—	96 ± 1	99 ± 2	101 ± 3	—
	3-APS	—	—	97 ± 1	103 ± 2	108 ± 2
Rise in BP induced by exogenous noradrenaline	GABA	—	—	92 ± 6	95 ± 6	116 ± 9
	R-(−)-Baclofen	—	89 ± 5	89 ± 9	88 ± 9	—
	S-(+)-Baclofen	—	—	101 ± 5	112 ± 6	133 ± 8
	Muscimol	—	89 ± 5	93 ± 3	108 ± 3	—
	3-APS	—	—	92 ± 6	109 ± 9	123 ± 15

Pithed rats received four injections of noradrenaline $0.3\,nmol\,kg^{-1}$ (S_1–S_4) at intervals of 7 min. GABA receptor agonists were injected i.v. in three increasing doses 5 min before S_2, S_3 and S_4. In the control series, the vehicle was injected instead (HCl $0.05\,mol\,l^{-1}$ for muscimol; saline for the other drugs). In each series of experiments, saline was injected 5 min before S_1. The ratios of the rise in blood pressure evoked by S_2, S_3 or S_4 over that evoked by S_1 were determined. To quantify the effects of the drugs on basal blood pressure, the ratios of the blood pressure immediately before S_2, S_3 or S_4 (t_2, t_3 and t_4) over that before S_1 (t_1) were determined. Results are given as % of controls; S_n/S_1 and t_n/t_1 values are given in Table 2 (for R-(−)-baclofen and its control) or not shown. 3-APS: 3-aminopropane sulphonic acid. Data shown are means ± s.e.mean of 4–12 experiments.
*$P < 0.05$ compared to the corresponding control (not shown).

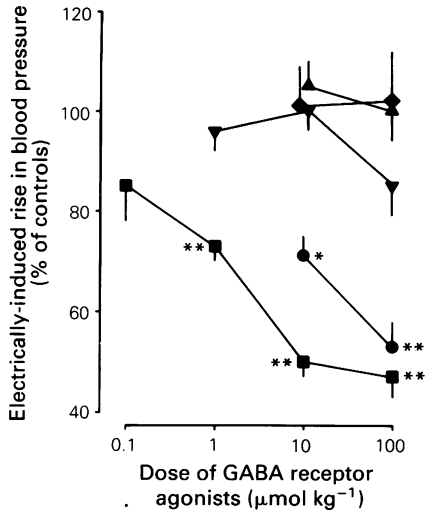

Figure 1 Effect of GABA (●), 3-aminopropane sulphonic acid (▲), muscimol (▼), R-(−)-baclofen (■) and S-(+)-baclofen (◆) on the electrically-induced rise in diastolic blood pressure. Four single electrical pulses of 30 V and 1 ms (S_1–S_4) were administered to pithed and vagotomized rats at intervals of 7 min. Saline was injected 5 min before S_1. The GABA receptor agonist under study was injected i.v. in three increasing doses 5 min before S_2, S_3 and S_4. The ratios of the rise in blood pressure evoked by S_2, S_3 or S_4 over that evoked by S_1 were determined; these ratios were expressed as percentages of the respective S_n/S_1 values in controls (vehicle injected i.v. 5 min before S_2, S_3 and S_4). The S_n/S_1 values obtained in the series with R-(−)-baclofen and in the corresponding control series (which also refers to S-(+)-baclofen, GABA and 3-aminopropane sulphonic acid) are given in Table 1. Points shown are means of 4–7 experiments and vertical lines represent s.e.mean. $*P < 0.02$, $**P < 0.005$ (compared to the corresponding controls).

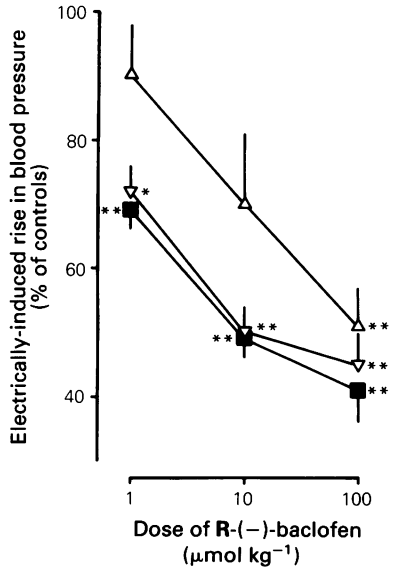

Figure 2 Effect of R-(−)-baclofen on the electrically-induced rise in diastolic blood pressure and interaction with GABA receptor antagonists. Four single electrical pulses of 30 V and 1 ms (S_1–S_4) were administered to pithed and vagotomized rats at intervals of 7 min. Bicuculline 10 μmol kg^{-1} (▽), 2-hydroxysaclofen 50 μmol kg^{-1} (△) or water (■) was injected i.v. 5 min before S_1. R-(−)-baclofen was injected i.v. in three increasing doses 5 min before S_2, S_3 and S_4. The ratios of the rise in blood pressure evoked by S_2, S_3 or S_4 over that evoked by S_1 were determined; these ratios were expressed as percentages of the respective S_n/S_1 values in controls (saline injected i.v. 5 min before S_2, S_3 and S_4). Points shown are means of 4–5 experiments and vertical lines represent s.e.mean. $*P < 0.02$, $**P < 0.005$ (compared to the corresponding controls).

2 ml kg^{-1} (bicuculline, 2-hydroxysaclofen) or 8 ml kg^{-1} (methohexitone).

Results

Basal diastolic blood pressure immediately before S_1 (first injection of noradrenaline or first electrical stimulation) was 47.7 ± 0.6 (n = 78) and 55.2 ± 1.5 (n = 18) in rats which had received saline or water 5 min beforehand, respectively. Basal blood pressure was not affected by the GABA$_A$-receptor antagonist bicuculline 10 μmol kg^{-1}, but slightly decreased by the GABA$_B$-receptor antagonist 2-hydroxysaclofen 50 μmol kg^{-1} (Table 1). GABA and GABA-receptor agonists (injected in increasing doses 5 min before S_2, S_3 and S_4) only slightly affected basal blood pressure (alteration ≤10%) or did not have any effect (Tables 2 and 3).

Noradrenaline 0.3 nmol kg^{-1} increased basal diastolic blood pressure by 7.5–27.5 mmHg. Subsequent injections administered at intervals of 7 min produced similar effects (Table 2 or not shown). The vasopressor response to noradrenaline was not affected by the GABA-receptor antagonists (Table 1) and by the GABA-receptor agonists at doses up to 100 μmol kg^{-1} (Tables 2 and 3). At 300 μmol kg^{-1}, GABA, S-(+)-baclofen and 3-aminopropane sulphonic acid tended to increase the effect of noradrenaline (Table 3).

Administration of a single electrical pulse of 30 V and 1 ms (between the pithing rod and an indifferent electrode placed dorsally) led to an increase in basal blood pressure of 10–30 mmHg in saline-treated rats. Subsequent stimuli administered at intervals of 7 min produced similar effects (Table 2 or not shown).

In the first series of experiments, the effects of GABA and GABA-receptor agonists on the electrically-induced rise in blood pressure were studied. (The effects of GABA, S-(+)-baclofen and 3-aminopropane sulphonic acid, each 300 μmol kg^{-1}, were not further considered since, at this high dose, the drugs interfered with the noradrenaline-induced rise in blood pressure (Table 3)). The electrically-induced vasopressor response was inhibited by GABA 10 and 100 μmol kg^{-1} in a dose-dependent manner (Figure 1). The GABA$_B$-receptor agonist R-(−)-baclofen even produced an inhibitory effect at a ten fold lower dose. The effect was nearly maximal at 10 μmol kg^{-1} (maximum response about 50–55%; Figure 1). Unlike its R-(−)-enantiomer, S-(+)-baclofen failed to inhibit the electrically-induced rise in blood pressure up to 100 μmol kg^{-1}; the same held true for the GABA$_A$-receptor agonist 3-aminopropane sulphonic acid (Figure 1). Another GABA$_A$-receptor agonist, muscimol, had no effect at 1 and 10 μmol kg^{-1} and tended to inhibit the electrically-induced rise in blood pressure at 100 μmol kg^{-1} (Figure 1).

In the second series of experiments, the interaction of R-(−)-baclofen with the GABA-receptor antagonists bicuculline and 2-hydroxysaclofen was studied. The dose-response curve of R-(−)-baclofen for its inhibitory effect on the electrically-induced vasopressor response was not affected by the GABA$_A$-receptor antagonist bicuculline 10 μmol kg^{-1}, but shifted to the right by the GABA$_B$-receptor antagonist 2-hydroxysaclofen 50 μmol kg^{-1} (by a factor of 10; Figure 2). Bicuculline and 2-hydroxysaclofen by themselves did not affect the electrically-induced rise in blood pressure (Table 1).

In the final series of experiments, the effect of R-(−)-baclofen 100 μmol kg^{-1} on the rise in blood pressure induced by a 30 s period of electrical stimulation (2 Hz, 30 V, 1 ms) was examined. The S_2/S_1 values in animals which received R-(−)-baclofen or saline were 0.83 ± 0.01 and 1.08 ± 0.03, respectively (n = 4 each; $P < 0.001$).

Discussion

It was the aim of the present study to examine the effects of GABA and related drugs (for review, see Bowery, 1989) on

the sympathetic nerves supplying the resistance vessels in the pithed rat. Both the effects of the drugs on the electrically-induced vasopressor response and their interaction with exogenously added noradrenaline were studied. The vasopressor response induced by a single electrical pulse appears to be solely due to release of catecholamines, since it was almost fully suppressed by prior administration of adrenoceptor antagonists at doses abolishing the vasopressor response to exogenously added noradrenaline without affecting that to ATP (unpublished results; under different schedules of electrical stimulation, ATP does contribute to the electrically-induced vasopressor response; Bulloch & McGrath, 1988; Schlicker *et al.*, 1989). The lack of effect of GABA and related drugs (at doses up to 100 μmol kg^{-1}) on the vasopressor response induced by exogenous noradrenaline allows us to use the electrically-induced end organ response as a parameter for the electrically-evoked transmitter release from sympathetic nerves.

The missing or very slight effects of the GABA-related drugs on basal blood pressure exclude the possibility that differences between the drugs with respect to their effects on the electrically-induced vasopressor response are related to differences in their effects on basal blood pressure (De Jonge *et al.*, 1983). The missing or very slight effect of **R**-(−)-baclofen on basal blood pressure, observed in the present model, is not discordant with the more marked hypotensive effect of low doses of racemic baclofen obtained in the anaesthetized rat (Chahl & Walker, 1980), since pithing abolishes the centrally driven sympathetic tone involved in the action of this drug (DeFeudis, 1983; Antonaccio, 1984; Bousquet *et al.*, 1985).

GABA decreased the electrically-induced vasopressor response, and its effect was mimicked by the $GABA_B$-receptor agonist **R**-(−)-baclofen, which was even more potent in this respect than GABA itself, whereas its **S**-(+)-enantiomer was inactive. The electrically-induced vasopressor response was not (or hardly) affected by the $GABA_A$-receptor agonists aminopropane sulphonic acid and muscimol. These results suggest the involvement of $GABA_B$-receptors in the depression of the electrically-induced rise in blood pressure. The rank order of potencies for the agonists **R**-(−)-baclofen, GABA and muscimol in the pithed rat is identical to that found under *in vitro* conditions in our previous study (in which $GABA_B$-receptors modulating 5-hydroxytryptamine release from rat brain cortex slices were examined; Schlicker *et al.*, 1984). This is most remarkable since, in the pithed rat, differences between the drugs might be influenced by differences in their pharmacokinetic properties.

Further support for the involvement of $GABA_B$-receptors in the effect of GABA comes from the results obtained with the antagonists; thus, the dose-response curve for **R**-(−)-baclofen was not affected by the $GABA_A$-receptor antagonist bicuculline but shifted to the right by 2-hydroxysaclofen, a recently characterized $GABA_B$ receptor antagonist (Curtis *et al.*, 1988; Kerr *et al.*, 1988), which is relatively potent and selective (no effect on, e.g., α-adrenoceptors, $GABA_A$ or muscarinic receptors; Kerr *et al.*, 1988; present study).

An inhibitory effect of **R**-(−)-baclofen on the neurogenic vasopressor response was also obtained when a 30 s period of electrical stimulation was administered at a frequency of 2 Hz. In this case, the extent of inhibition was lower than under the standard condition (single pulse). The difference in the degree of inhibition was not unexpected, since receptor-mediated modulation of transmitter release is highly dependent on the conditions used for stimulation (Starke, 1977). The differential inhibitory effect of **R**-(−)-baclofen is compatible with the view that the $GABA_B$-receptors considered here are located presynaptically on the postganglionic sympathetic nerve fibres, although a presynaptic location on the preganglionic sympathetic nerve fibres is conceivable as well.

The finding that the inhibitory effect of **R**-(−)-baclofen is detectable at 2 Hz (i.e. at a frequency normally occurring in the autonomic nerves) provides evidence that the $GABA_B$-receptors might be activated under physiological conditions. Although GABAergic neurones have so far not been detected in the vascular wall (unlike in the myenteric plexus of the guinea-pig, Jessen *et al.*, 1983), glutamic acid decarboxylase, the enzyme that synthesizes GABA, has been shown to occur in various blood vessels (Krause, 1986).

Even if the $GABA_B$-receptors under investigation should not have a physiological role, they might be of clinical relevance, provided they are also detectable in human resistance vessels. Firstly, the decrease in blood pressure which sometimes occurs as a side-effect on treatment with baclofen might be related to the activation of presynaptic $GABA_B$-receptors in the resistance vessels, although there is much evidence that central $GABA_B$-receptors are involved in this side-effect (Sill *et al.*, 1986; see also above). Secondly, the characterized $GABA_B$-receptors might become the target for future antihypertensive drugs. For further investigation of both points, it would be interesting to examine the blood pressure effects of $GABA_B$-receptor agonists which, unlike baclofen, do not cross the blood-brain barrier.

In conclusion, the present study shows that $GABA_B$-receptors are also detectable in vessels of the rat and, in particular, that they occur in resistance vessels. These $GABA_B$-receptors, which most probably are located presynaptically on the postganglionic sympathetic nerve fibres, might be involved in the modulation of blood pressure and might become another target for antihypertensive drugs.

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