Noradrenaline release in the rat vena cava is inhibited by γ -aminobutyric acid via GABA_B receptors but not affected by histamine

Dörthe Schneider,¹ Eberhard Schlicker,² Barbara Malinowska & Gerhard Molderings

Institut für Pharmakologie und Toxikologie, Rheinische Friedrich-Wilhelms-Universität Bonn, Reuterstr. 2 b, D-5300 Bonn 1, Germany

1 Segments of the rat vena cava preincubated with [³H]-noradrenaline were superfused with [³H]-noradrenaline-free solution containing desipramine and corticosterone and the effects of γ -aminobutyric acid (GABA) receptor ligands and of histamine on tritium overflow evoked by transmural electrical stimulation were studied.

2 GABA inhibited, and histamine failed to affect, the electrically (0.66 Hz) evoked tritium overflow both in the absence and presence of rauwolscine (which was present in the superfusion medium in the subsequent experiments). The effect of GABA was less pronounced at a stimulation frequency of 2 Hz.

3 The inhibitory effect of GABA (pIC₃₅ 5.83) on the electrically (0.66 Hz) evoked overflow was mimicked by the GABA_B receptor agonist, R-(−)-baclofen (6.07) and less potently by S-(+)-baclofen (3.30) and the GABA_A receptor agonist, muscimol (3.70). The concentration-response curve of GABA was shifted to the right by the GABA_B receptor antagonist, CGP 35348 (P-(3-aminopropyl)-P-diethoxymethyl-phosphinic acid; apparent pA₂ 4.76), but not affected by the GABA_A receptor antagonist, (−)-bicuculline methiodide 100 μ mol l^{−1}. Given alone, (−)-bicuculline methiodide slightly increased, and CGP 35348 did not affect, the evoked overflow.

4 The effect of GABA in veins from rats treated for 14 days with RS-baclofen (10 mg kg^{−1}, i.p. once daily) did not differ from that in veins from rats which received the vehicle instead. The effect of GABA also did not differ in veins from rats treated once either with RS-baclofen or with its vehicle.

5 The results suggest that the postganglionic sympathetic nerve fibres in the rat vena cava are endowed with presynaptic GABA_B receptors, but not with receptors for histamine. (−)-Bicuculline methiodide increases noradrenaline release by a mechanism not related to GABA_A, GABA_B, α_2 -receptors or noradrenaline uptake.

Keywords: Rat vena cava; postganglionic sympathetic neurones; noradrenaline release; γ -aminobutyric acid; presynaptic GABA_B receptors; histamine; baclofen; (−)-bicuculline methiodide; CGP 35348; muscimol

Introduction

Presynaptic GABA_B receptors have been shown to be present on postganglionic sympathetic nerve fibres innervating vessels of the rabbit (Starke & Weitzell, 1980; Anwar & Mason, 1982; Manzini *et al.*, 1985) and the goat (Miranda *et al.*, 1989). Recently, we found that the neurogenic vasopressor response in the pithed rat is inhibited by γ -aminobutyric acid (GABA) via GABA_B receptors (Kohlenbach & Schlicker, 1990) and that the maximum extent of inhibition (50%) is at least as high as that in the studies on rabbit and goat vessels quoted above. Thus, it was tempting to examine the possible occurrence of GABA_B receptors also in an *in vitro* vascular preparation of the rat. We used the superfused vena cava in which a series of presynaptic receptors has been characterized in previous papers from our group (Göthert & Kollécker, 1986; Göthert *et al.*, 1986; Molderings *et al.*, 1987), thus allowing a direct comparison between the effects of GABA and other neurotransmitters on noradrenaline release.

In addition, the effect of histamine on noradrenaline release in the superfused rat vena cava was studied. So far, an H₃ receptor-mediated effect of histamine on noradrenaline release from blood vessels has not been described. However, the existence of presynaptic H₃ receptors on postganglionic sympathetic nerve fibres innervating vessels is suggested by the findings of Ishikawa & Sperelakis (1987) who showed that histamine, via H₃ receptors, inhibited the amplitude of the elec-

trically evoked excitatory junction potentials in the guinea-pig mesenteric artery.

Preliminary accounts of the present results were given to the 7th International Symposium on Vascular Neuroeffector Mechanisms (Schlicker *et al.*, 1990a) and to the 32nd Spring Meeting of the Deutsche Gesellschaft für Pharmakologie und Toxikologie (Schneider, 1991).

Methods

The cranial segment of the inferior vena cava of male Wistar rats was dissected and ligated at both ends. Subsequently, the vein was incubated for 30 min in 1.5 ml physiological salt solution (37°C, composition see below) containing (−)-[ring-2, 5, 6-³H] noradrenaline 0.1 μ mol l^{−1} (specific activity 42.1–43.7 Ci mmol^{−1}) and then mounted vertically in an organ bath (tension adjusted to 0.5 g) between two parallel platinum electrodes (1.5 cm long). The adventitial surface of the vein was superfused with [³H]-noradrenaline-free physiological salt solution of 37°C at a rate of 2 ml min^{−1}. The composition of the solution was (mmol l^{−1}): NaCl 118, Na₂HPO₄ 1.2, NaHCO₃ 25.0, KCl 4.7, CaCl₂ 1.6, MgSO₄ 1.2, glucose 11, ascorbic acid 0.3, Na₂EDTA 0.03 (aerated with 95% O₂ and 5% CO₂). Throughout superfusion, the solution contained desipramine 0.6 μ mol l^{−1} and corticosterone 40 μ mol l^{−1} for inhibition of neuronal and extraneuronal noradrenaline uptake, respectively. Five periods of electrical stimulation (240 rectangular pulses of 100 mA and 0.3 ms at 0.66 or 2 Hz) were applied to each vein after 93 (S₁), 117 (S₂), 141 (S₃), 165 (S₄) and 189 (S₅) min of superfusion. The superfusate was collected

¹ Author for correspondence.

² Permanent address: Zakład Farmakodynamiki, Akademia Medyczna, Mickiewicza 2 C, 15-222 Białystok 8, Poland.

in 3 or 6 min fractions. The radioactivity in the superfusate samples and veins was determined by liquid scintillation counting.

Calculations and statistics

Tritium efflux was expressed as a fraction of the tritium present in the vein at the start of the respective collection period (fractional rate of tritium efflux). Basal ³H efflux was expressed as the ratio of the fractional rate during the collection period immediately before S₃ (t₃), S₄ (t₄) or S₅ (t₅) over that immediately before S₂ (t₂). Stimulation-evoked overflow of tritium was calculated by subtraction of the basal efflux (assumed to decrease linearly from the collection period before to that 12–15 min after onset of stimulation) from the total efflux during the 12 min subsequent to the onset of stimulation. Evoked ³H overflow was given as a percentage of tissue tritium at the start of stimulation, and the ratios of the overflow evoked by S₃, S₄ or S₅ over that evoked by S₂ were determined. In order to determine the effects of histamine and GABA receptor ligands (*in vitro*) on basal and stimulation-evoked tritium overflow, the ratios t₃/t₂, t₄/t₂ or t₅/t₂ and S₃/S₂, S₄/S₂ or S₅/S₂ obtained in the presence of the drug under study were compared to the corresponding ratios obtained in its absence. For determination of the effects of rauwolsine (*in vitro*) and of RS-baclofen (*in vivo*), the fractional rate of tritium efflux during t₂ or the tritium overflow evoked by S₂ in veins exposed to the drug under study was compared to the respective parameter in untreated veins.

Results are given as means ± s.e.mean. Student's *t* test for unpaired data was used for comparison of mean values. If two or more experimental series were compared to the same control series, the *t* test was subjected to Bonferroni's procedure. The apparent pA₂ value of CGP 35348 was calculated according to formula 4 of Furchgott (1972).

Drugs used

γ-Aminobutyric acid (GABA), (–)-bicuculline methiodide, corticosterone, muscimol (Sigma, Munich, F.R.G.); R(–), S(+)-baclofen hydrochloride, RS-baclofen, CGP 35348 (P-(3-

aminopropyl)-P-diethoxymethyl-phosphinic acid), desipramine hydrochloride (CIBA-Geigy, Wehr, F.R.G. or Basle, Switzerland); histamine dihydrochloride (Merck, Darmstadt, F.R.G.); rauwolsine hydrochloride (Roth, Karlsruhe, F.R.G.).

Results

Basal tritium efflux

For quantification of the basal tritium efflux, the fractional rate of ³H efflux during t₂ or the ratios t₃/t₂, t₄/t₂ and t₅/t₂ (t_n/t₂) were determined (for further details, see Methods). The fractional rate of ³H efflux during t₂ was not affected by rauwolsine 1 μmol l⁻¹ *in vitro* or by *in vivo* treatment of rats with RS-baclofen (or its vehicle; Table 1). The ratios t₃/t₂, t₄/t₂ and t₅/t₂ were 0.96 ± 0.03, 0.88 ± 0.02 and 0.85 ± 0.03, respectively, in control experiments (i.e. GABA receptor ligands and histamine absent) performed on vascular segments exposed to rauwolsine (*n* = 8–9); these ratios were not changed by omission of rauwolsine from the superfusion medium or by RS-baclofen (or its vehicle) administered *in vivo* (results not shown). The ratio t_n/t₂ was not affected by histamine or the GABA receptor ligands in the whole concentration-range (results not shown).

Electrically evoked tritium overflow

To quantify the electrically evoked tritium overflow, the ³H overflow evoked by S₂ or the ratios S₃/S₂, S₄/S₂ and S₅/S₂ (S_n/S₂) were determined (for further details, see Methods). Tritium overflow evoked by S₂ was markedly increased by rauwolsine 1 μmol l⁻¹, but not affected by *in vivo* treatment of rats with RS-baclofen (or its vehicle; Table 1). The ratios S₃/S₂, S₄/S₂ and S₅/S₂ were 1.03 ± 0.04, 1.01 ± 0.06 and 0.93 ± 0.07, respectively, in 7–9 controls (histamine and GABA receptor ligands absent) superfused in the presence of rauwolsine 1 μmol l⁻¹. Similar values were obtained for control experiments on vascular segments superfused in the absence of rauwolsine or on preparations obtained from rats which were pretreated with RS-baclofen (or its vehicle; Table

Table 1 Effects of drugs administered *in vivo* (RS-baclofen) or *in vitro* (rauwolsine) on the basal and electrically (0.66 Hz) evoked tritium overflow and on the GABA-mediated inhibition of the latter in rat vena cava segments preincubated with [³H]-noradrenaline

Compound administered in vivo	Rauwolsine (μmol l ⁻¹) administered in vitro	Basal ³ H efflux (t ₂) ^a expressed as fractional rate (% × min ⁻¹) ^b	Evoked ³ H overflow (S ₂) expressed as % of tissue tritium ^c	Evoked ³ H overflow (S ₃ /S ₂)	
				No GABA	GABA (10 μmol l ⁻¹)
—	—	0.20 ± 0.01	1.42 ± 0.11	1.15 ± 0.08	0.51 ± 0.06†
—	1 ^d	0.19 ± 0.01 ^e	3.65 ± 0.27 ^{f,*}	0.93 ± 0.07	0.40 ± 0.04†
Vehicle, 1 injection ^g	1	0.16 ± 0.01	4.81 ± 0.62	0.96 ± 0.10	0.41 ± 0.05†
RS-baclofen (10 mg kg ⁻¹) 1 injection ^g	1	0.19 ± 0.01	3.74 ± 0.45	1.08 ± 0.05	0.43 ± 0.06†
Vehicle, 14 injections ^h	1	0.18 ± 0.02	3.65 ± 0.26	0.93 ± 0.05	0.46 ± 0.04†
RS-baclofen (10 mg kg ⁻¹) 14 injections ^h	1	0.18 ± 0.01	4.57 ± 0.40	0.91 ± 0.07	0.51 ± 0.03†

The veins were superfused with [³H]-noradrenaline-free physiological salt solution containing desipramine 0.6 μmol l⁻¹ and corticosterone 40 μmol l⁻¹ throughout superfusion and GABA in three increasing concentrations 9 min before and during S₃, S₄ and S₅. Five 6 min periods of transmural electrical stimulation (S₁–S₅) were applied in each preparation (after 93, 117, 141, 165 and 189 min of superfusion) and the ratios of the ³H overflow evoked by S₃, S₄ and S₅ over that evoked by S₂ were determined. Only the ratios S₃/S₂ obtained in the absence or presence of GABA 10 μmol l⁻¹ are shown. Means ± s.e.mean of 4–28 experiments.

* *P* < 0.001, compared to the value without rauwolsine, † *P* < 0.005, compared to the corresponding control ('NO GABA').

^a t₂ represents the 3-min period of superfusate sampling immediately before S₂.

^b Efflux of tritium per min, expressed as fraction of tissue tritium.

^c Evoked overflow in excess of basal efflux.

^d Present in the superfusion fluid from 90 min of superfusion onward.

^e Corresponding to 0.18 ± 0.02 nCi min⁻¹.

^f Corresponding to 2.14 ± 0.27 nCi.

^g Rats received one i.p. injection of RS-baclofen or its vehicle (water) 24 hours before decapitation.

^h Rats received i.p. injections of RS-baclofen or its vehicle (water) for 14 days (once daily); the last injection was administered 24 h before decapitation.

1 or not shown). In the following paragraphs, the S_3/S_2 , S_4/S_2 and S_5/S_2 values obtained in the presence of GABA receptor ligands and histamine relative to the respective control values are considered.

GABA inhibited the electrically (0.66 Hz) evoked tritium overflow in a concentration-dependent manner; the extent of inhibition was virtually the same when rauwolscline $1 \mu\text{mol l}^{-1}$ was present in, or omitted from, the superfusion medium (Figure 1a). Histamine failed to affect the evoked overflow both in the absence and presence of the α_2 -adrenoceptor antagonist (Figure 1a). Rauwolscline was present in each of the experiments to be described now. The inhibitory effect of GABA was less pronounced when the frequency of stimulation used to evoke tritium overflow was 2 instead of 0.66 Hz (Figure 1b).

The inhibitory effect of GABA on the electrically (0.66 Hz) evoked tritium overflow was mimicked by the GABA_B receptor agonist, R(-)-baclofen and, less potently, by its S-(+)-enantiomer and by the GABA_A receptor agonist, muscimol

Figure 1 Effect of γ -aminobutyric acid (GABA) and/or histamine on the electrically evoked tritium overflow from rat vena cava segments preincubated with [^3H]-noradrenaline (a) in the presence or absence of rauwolscline and (b) with different frequencies of stimulation. The veins were superfused with [^3H]-noradrenaline-free physiological salt solution containing desipramine $0.6 \mu\text{mol l}^{-1}$ and corticosterone $40 \mu\text{mol l}^{-1}$. Five periods of transmural electrical stimulation (S_1 – S_5 ; 240 pulses each) were applied at 0.66 or 2 Hz. The ratios of the ^3H overflow evoked by S_3 , S_4 or S_5 over that evoked by S_2 are given, expressed as percentages of the ratios obtained in the respective control experiments (not shown). GABA or histamine was added to the superfusion fluid at increasing concentrations 9 min before and during S_3 , S_4 and S_5 ; rauwolscline $1 \mu\text{mol l}^{-1}$ was present in the superfusion fluid from 3 min before S_1 onward. Explanation of symbols: circles, GABA; squares, histamine; open symbols, no rauwolscline; filled symbols, rauwolscline $1 \mu\text{mol l}^{-1}$; solid line, 0.66 Hz; broken line, 2 Hz. Means of 4–18 experiments with s.e.mean shown by vertical lines. * $P < 0.05$; ** $P < 0.005$ (compared to the corresponding ratios in the respective control experiments).

Figure 2 Effect of γ -aminobutyric acid (GABA) receptor agonists on the electrically (0.66 Hz) evoked tritium overflow from rat vena cava segments preincubated with [^3H]-noradrenaline (a) and interaction of GABA with GABA receptor antagonists (b). The veins were superfused with [^3H]-noradrenaline-free physiological salt solution containing desipramine $0.6 \mu\text{mol l}^{-1}$ and corticosterone $40 \mu\text{mol l}^{-1}$. Five 6 min periods of transmural electrical stimulation were applied (S_1 – S_5). The ratios of the ^3H overflow evoked by S_3 , S_4 or S_5 over that evoked by S_2 are given, expressed as percentages of the ratios obtained in the respective control experiments (not shown). Rauwolscline $1 \mu\text{mol l}^{-1}$ and, if relevant, the GABA_A receptor antagonist, (–)-bicuculline methiodide $100 \mu\text{mol l}^{-1}$ or the GABA_B receptor antagonist, CGP 35348 $100 \mu\text{mol l}^{-1}$ were present in the superfusion fluid from 3 min before S_1 onward. GABA (●: in the presence of bicuculline, ○: in the presence of CGP 35348, ◐: in the absence of either antagonist), R(-)-baclofen (▼), S-(+)-baclofen (▲) or muscimol (◆) was added to the superfusion fluid at increasing concentrations 9 min before and during S_3 , S_4 and S_5 . Means of 4–18 experiments; vertical lines show s.e.mean. * $P < 0.05$; ** $P < 0.001$ (compared to the corresponding ratios in the respective control experiments).

(Figure 2a). The maximum degree of inhibition obtainable with GABA or R(-)-baclofen was about 70%. Since an inhibition by 35% may, therefore, represent the half-maximum effect, IC_{35} values were determined from Figure 2a to quantify the potencies of the four agonists (in the case of muscimol, the IC_{35} value was extrapolated). IC_{35} values for R(-)-baclofen, GABA, muscimol and S-(+)-baclofen were 0.85, 1.48, 200 and $500 \mu\text{mol l}^{-1}$, respectively. The effect of GABA $10 \mu\text{mol l}^{-1}$ was not affected by the GABA_A receptor antagonist, (–)-bicuculline methiodide $100 \mu\text{mol l}^{-1}$, whereas the GABA_B receptor antagonist, CGP 35348 $100 \mu\text{mol l}^{-1}$ shifted to the right the concentration-response curve of GABA (Figure 2b; apparent pA_2 value: 4.76). Given alone, (–)-bicuculline methiodide 30 and $100 \mu\text{mol l}^{-1}$ increased the evoked overflow by 35 and 36%, respectively, whereas CGP 35348, in the same concentration-range, had no effect (Table 2).

In another series of experiments, we examined whether acute or chronic treatment of rats with RS-baclofen affects the

Table 2 Effects of (–)-bicuculline methiodide and CGP 35348 on the electrically (0.66 Hz) evoked tritium overflow from rat vena cava segments preincubated with [^3H]-noradrenaline

GABA receptor antagonist	Electrically evoked ^3H overflow (% of controls)		
	S_3/S_2	S_4/S_2	S_5/S_2
(–)-Bicuculline methiodide ^a	118 ± 11	135 ± 12*	136 ± 11*
CGP 35348 ^a	103 ± 3	98 ± 5	95 ± 4

The veins were superfused with [^3H]-noradrenaline-free physiological salt solution containing desipramine $0.6 \mu\text{mol l}^{-1}$ and corticosterone $40 \mu\text{mol l}^{-1}$. Five 6 min periods of transmural electrical stimulation were applied to each preparation (S_1 – S_5). The ratios of the ^3H overflow evoked by S_3 , S_4 or S_5 over that evoked by S_2 are given, expressed as percentages of the ratios obtained in the respective controls (not shown). Rauwolscline $1 \mu\text{mol l}^{-1}$ was present in the superfusion medium from 3 min before S_1 onward; the GABA receptor antagonist under study was added to the medium in three increasing concentrations 9 min before and during S_3 , S_4 and S_5 . Means ± s.e.mean of 6–13 experiments.

* $P < 0.02$ (compared to the corresponding control).

^a Concentration during S_3 , S_4 and S_5 : 10, 30 and $100 \mu\text{mol l}^{-1}$, respectively.

effect of GABA $0.1\text{--}10\ \mu\text{mol l}^{-1}$ on the electrically (0.66 Hz) evoked tritium overflow. Treatment of animals with RS-baclofen for 14 days did not change the effect of GABA on tritium overflow in isolated vena cava segments compared to preparations from animals which had received the vehicle instead (Table 1 or not shown; $n = 7\text{--}10$). There was also no difference in the effect of GABA between veins from animals which received only one injection of RS-baclofen or of its vehicle (Table 1 or not shown; $n = 4\text{--}6$).

Discussion

The electrically evoked tritium overflow from segments of the rat vena cava (which represents quasi-physiological noradrenaline release; Göthert *et al.*, 1986) was inhibited by GABA, but not affected by histamine. When the effects of both drugs were studied in the presence of rauwolscine, virtually the same results were obtained as in its absence. Thus, the α_2 -adrenoceptor antagonist could be included in the superfusion fluid in order to increase stimulation-evoked noradrenaline release. With respect to the experiments with histamine, there was also another reason to add a drug with α_2 -adrenolytic properties to the superfusion medium. Thus, the H_3 -receptor-mediated inhibitory effect of histamine on noradrenaline release in superfused rat brain cortex slices and pig retina discs was unmasked or at least increased by rauwolscine or the unselective α -adrenoceptor antagonist, phentolamine but not by the α_1 -adrenoceptor antagonist, prazosin (Schlicker *et al.*, 1989; 1990b; unpublished results). The failure of histamine to inhibit noradrenaline release in the present model suggests that the postganglionic sympathetic nerve fibres innervating the rat vena cava are not endowed with presynaptic H_3 receptors.

Both the data obtained with GABA receptor agonists and antagonists demonstrate that the effect of GABA is mediated via GABA_B receptors. Thus, the relative potencies of the GABA receptor agonists in the present model closely resemble their relative affinities for GABA_B binding sites on rat whole brain synaptic membranes (Bowery *et al.*, 1983). The relative potencies (IC_{35} value of R(-)-baclofen divided by the IC_{35} value of the respective GABA receptor agonist) for R(-)-baclofen, GABA, muscimol and S-(+)-baclofen were 1, 0.57, 0.004 and 0.002, respectively, and the relative affinities (IC_{50} of R(-)-baclofen divided by IC_{50} of the respective GABA receptor agonist) were 1, 1.02, 0.003 and 0.001, respectively. The effect of GABA in the present model was not affected by a high concentration of the GABA_A receptor antagonist, (-)-bicuculline methiodide, whereas the recently characterized GABA_B receptor antagonist, CGP 35348 (Olpe *et al.*, 1990) produced a rightward shift of the concentration-response curve of GABA. The apparent pA_2 value (4.76) closely resembles its pIC_{50} value for the inhibition of GABA_B binding in rat brain cortex membranes (4.47; Olpe *et al.*, 1990; determined with the agonist ligand [3H]-aminopropylphosphinic acid); it was almost identical to its pA_2 value against RS-baclofen for its inhibitory effect on the field stimulation-induced twitch response in the rat vas deferens (5.0; Hills *et al.*, 1991a) and only slightly lower than its pA_2 value against the GABA_B receptor agonist, 3-aminopropylphosphinic acid, for its inhibitory effect on the electrically induced contraction in the rat anococcygeus muscle (5.38; Hills *et al.*, 1991b). The GABA_B receptor-mediated effect of GABA in the rat vena cava was attenuated when the frequency of stimulation was increased from 0.66 to 2 Hz; a frequency-dependence was also obtained for effects mediated via GABA_B autoreceptors (Raiteri *et al.*, 1989; Baumann *et al.*, 1990) and heteroreceptors (mediating inhibition of 5-hydroxytryptamine (5-HT) release; Schlicker *et al.*, 1984) in the rat brain cortex.

The inhibitory effect of GABA on noradrenaline release in the rat vena cava could be directly compared to the effects of two other neurotransmitters which have been examined under almost identical conditions (stimulation frequency of 0.66 Hz).

Thus, the maximum extent of inhibition produced by GABA (by 70%) was only slightly less than that produced by noradrenaline (by 80%; effect mediated via α_2 -adrenoceptors; Göthert & Kollacker, 1986; Molderings & Göthert, unpublished results) and markedly higher than that produced by 5-HT (by 30%; effect mediated via 5-HT_{1B} receptors; Göthert *et al.*, 1986; Molderings *et al.*, 1987). The maximum effect shown by R(-)-baclofen in the present model was identical to that at the GABA_B autoreceptor in rat brain cortex slices (stimulation frequency: 0.5 Hz; Waldmeier *et al.*, 1988). Furthermore, the potency of R(-)-baclofen in the present model (pIC_{35} 6.07) was only slightly lower than at the GABA_B autoreceptor in the rat brain (pIC_{50} 6.43; stimulation frequency: 0.25 Hz; Baumann *et al.*, 1990). With respect to the marked effect of GABA in the vena cava both in terms of the maximum degree of inhibition and the potency, the question arises whether GABA might also act on these receptors *in vivo*. However, the results obtained with CGP 35348 are not in favour of the idea that the GABA_B heteroreceptors in the present model are activated tonically by endogenous GABA. In this case, one would have expected that CGP 35348, given alone, would increase noradrenaline release by interruption of the inhibition produced by endogenous GABA (e.g., a facilitation of noradrenaline release in the present model was obtained with rauwolscine suggesting that the presynaptic α_2 -adrenoceptors are tonically activated by endogenous noradrenaline). Although a physiological function of the GABA_B receptors in the rat vena cava cannot be postulated on the basis of the present study, one has to consider that, besides the sympathetic nervous system, there are other sources for GABA (e.g., blood cells, endocrine and exocrine cells; for review, see Erdő & Wolff, 1990) which might provide GABA *in vivo*.

Unlike CGP 35348, (-)-bicuculline methiodide increased noradrenaline release. This effect is not related to the blockade of GABA_A receptors which could not be detected in the present study. The facilitatory effect of (-)-bicuculline methiodide on noradrenaline release is also not due to blockade of neuronal or extraneuronal uptake or α_2 -adrenoceptors since the relevant experiments were carried out in the presence of desipramine, corticosterone and rauwolscine, respectively. The effect of (-)-bicuculline methiodide is reminiscent of its facilitatory effect on the electrically induced contractions in the guinea-pig ileum (Bartolini *et al.*, 1990) and might be related to direct nonsynaptic actions on neuronal membranes shown for high concentrations of bicuculline (Heyer *et al.*, 1981).

Finally, we examined whether long-term treatment of rats with RS-baclofen produces a down-regulation of the GABA_B receptors. However, treatment of rats with RS-baclofen 10 mg kg^{-1} (i.p., once daily) for 14 days (or for 1 day) failed to affect the inhibitory effect of GABA in the vena cava. In two previous studies on mice, chronic treatment with RS-baclofen according to the same protocol had reduced the inhibitory effect of baclofen on 5-HT release (Gray *et al.*, 1986) and the number of GABA_B binding sites in preparations of the cerebral cortex (Suzdak & Gianutsos, 1986).

In conclusion, the present study extends our previous one in the pithed rat (Kohlenbach & Schlicker, 1990) in several respects. First, release of the neurotransmitter was determined instead of end organ responses; second, the present study shows that the GABA_B receptors are located presynaptically on the postganglionic sympathetic nerve fibres; third, GABA_B receptors are not restricted to resistance vessels, but also occur in the vena cava. These GABA_B receptors do not appear to be activated tonically by endogenous GABA and did not show down-regulation after chronic treatment of rats with RS-baclofen.

B.M. is recipient of a research fellowship provided by the Alexander von Humboldt-Stiftung (Bonn, F.R.G.). We wish to thank CIBA-Geigy for gifts of RS-baclofen and its enantiomers, of CGP 35348 and of desipramine.

References

- ANWAR, N. & MASON, D.F.J. (1982). Two actions of γ -aminobutyric acid on the responses of the isolated basilar artery of the rabbit. *Br. J. Pharmacol.*, **75**, 177–181.
- BARTOLINI, A., GIOTTI, A., GIULIANI, S., MALMBERG-AIELLO, P. & PATACCHINI, R. (1990). Bicuculline actions on isolated rat atria, mouse vas-deferens and guinea-pig ileum are unrelated to GABA_A receptor blockade. *Gen. Pharmacol.*, **21**, 277–284.
- BAUMANN, P.A., WICKI, P., STIERLIN, C. & WALDMEIER, P.C. (1990). Investigations on GABA_B receptor-mediated autoinhibition of GABA release. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **341**, 88–93.
- BOWERY, N.G., HILL, D.R. & HUDSON, A.L. (1983). Characteristics of GABA_B receptor binding sites on rat whole brain synaptic membranes. *Br. J. Pharmacol.*, **78**, 191–206.
- ERDŐ, S.L. & WOLFF, J.R. (1990). γ -Aminobutyric acid outside the mammalian brain. *J. Neurochem.*, **54**, 363–372.
- FURCHGOTT, R.F. (1972). The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In *Handbook of Experimental Pharmacology*, ed. Blaschko, H. & Muscholl E. pp. 283–335. Berlin, Heidelberg, New York: Springer Verlag.
- GÖTHERT, M. & KOLLECKER, P. (1986). Subendothelial β_2 -adrenoceptors in the rat vena cava: Facilitation of noradrenaline release via local stimulation of angiotensin II synthesis. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **334**, 156–165.
- GÖTHERT, M., SCHLICKER, E. & KOLLECKER, P. (1986). Receptor-mediated effects of serotonin and 5-methoxytryptamine on noradrenaline release in the rat vena cava and in the heart of the pithed rat. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **332**, 124–130.
- GRAY, J.A., GREEN, A.R. & MOLYNEUX, S.G. (1986). Effect of baclofen on the release of endogenous 5-HT from mouse cortex slices. *Br. J. Pharmacol.*, **87**, 149P.
- HEYER, E.J., NOWAK, L.M. & MACDONALD, R.L. (1981). Bicuculline: a convulsant with synaptic and nonsynaptic actions. *Neurology*, **31**, 1381–1390.
- HILLS, J.M., LARKIN, M.M. & HOWSON, W. (1991a). A comparison of the relative activities of a number of GABA_B antagonists in the isolated vas deferens of the rat. *Br. J. Pharmacol.*, **102**, 631–634.
- HILLS, J.M., SELLERS, A.J., MISTRY, J., BROEKMAN, M. & HOWSON, W. (1991b). Phosphinic acid analogues of GABA are antagonists at the GABA_B receptor in the rat anococcygeus. *Br. J. Pharmacol.*, **102**, 5–6.
- ISHIKAWA, S. & SPERELAKIS, N. (1987). A novel class (H₃) of histamine receptors on perivascular nerve terminals. *Nature*, **327**, 158–160.
- KOHLBACH, A. & SCHLICKER, E. (1990). GABA_B receptor-mediated inhibition of the neurogenic vasopressor response in the pithed rat. *Br. J. Pharmacol.*, **100**, 365–369.
- MANZINI, S., MAGGI, C.A. & MELI, A. (1985). Inhibitory effect of GABA on sympathetic neurotransmission in rabbit ear artery. *Arch. Int. Pharmacodyn.*, **273**, 100–109.
- MIRANDA, F.J., TORREGROSA, G., SALOM, J.B., CAMPOS, V., ALBADI, J.A. & ALBORCH, E. (1989). Inhibitory effect of GABA on cerebrovascular sympathetic neurotransmission. *Brain Res.*, **492**, 45–52.
- MOLDERINGS, G.J., FINK, K., SCHLICKER, E. & GÖTHERT, M. (1987). Inhibition of noradrenaline release in the rat vena cava via presynaptic 5-HT_{1B} receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **336**, 245–250.
- OLPE, H.R., KARLSSON, G., POZZA, M.F., BRUGGER, F., STEINMANN, M., VAN RIEZEN, H., FAGG, G., HALL, R.G., FROESTL, W. & BITTIGER, H. (1990). CGP 35348: a centrally active blocker of GABA_B receptors. *Eur. J. Pharmacol.*, **187**, 27–38.
- RAITERI, M., BONANNO, G. & FEDELE, E. (1989). Release of γ -[³H] aminobutyric acid (GABA) from electrically stimulated rat cortical slices and its modulation by GABA_B autoreceptors. *J. Pharmacol. Exp. Ther.*, **250**, 648–653.
- SCHLICKER, E., CLASSEN, K. & GÖTHERT, M. (1984). GABA_B receptor-mediated inhibition of serotonin release in the rat brain. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **326**, 99–105.
- SCHLICKER, E., FINK, K., HINTERTHANER, M. & GÖTHERT, M. (1989). Inhibition of noradrenaline release in the rat brain cortex via presynaptic H₃ receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **340**, 633–638.
- SCHLICKER, E., KOHLBACH, A. & SCHNEIDER, D. (1990a). Inhibition of the neurogenic vasopressor response in the pithed rat via GABA_B receptors. *Blood Vessels*, **27**, 56.
- SCHLICKER, E., SCHUNACK, W. & GÖTHERT, M. (1990b). Histamine H₃ receptor-mediated inhibition of noradrenaline release in pig retina discs. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **342**, 497–501.
- SCHNEIDER, D. (1991). Noradrenaline release in the rat vena cava is inhibited by GABA via GABA_B receptors but not affected by histamine. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **343** (Suppl), R 94.
- STARKE, K. & WEITZELL, R. (1980). γ -Aminobutyric acid and postganglionic sympathetic transmission in the pulmonary artery of the rabbit. *J. Auton. Pharmacol.*, **1**, 45–51.
- SUZDAK, P.D. & GIANUTSOS, G. (1986). Effect of chronic imipramine or baclofen on GABA_B binding and cyclic AMP production in cerebral cortex. *Eur. J. Pharmacol.*, **131**, 129–133.
- WALDMEIER, P.C., WICKI, P., FELDTRAUER, J.J. & BAUMANN, P.A. (1988). Potential involvement of a baclofen-sensitive autoreceptor in the modulation of the release of endogenous GABA from rat brain slices *in vitro*. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **337**, 289–295.

(Received March 11, 1991

Revised June 12, 1991

Accepted June 20, 1991)