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TWO ACTIONS OF γ-AMINOBUTYRIC ACID ON THE RESPONSES OF THE ISOLATED BASILAR ARTERY FROM THE RABBIT

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1 In the isolated basilar artery of the rabbit, γ-aminobutyric acid (GABA) ($ED_{50} \pm$ s.e.mean, $2.4 \pm 1.1 \times 10^{-5}$ M) produced a relaxation, if the tone had been increased with 5-hydroxytryptamine (5-HT).

2 3-Aminoproprane sulphonic acid (3-APS) produced a similar, but smaller relaxation, while baclofen had no effect. The relaxation produced by GABA was inhibited by bicuculline.

3 Transmural electrical stimulation produced a reproducible contraction of the isolated basilar artery. In 9 out of 14 preparations GABA ($ED_{50} \pm$ s.e.mean, $5.6 \pm 2.1 \times 10^{-7}$ M) caused a reduction of the response, with a maximum of $49.2 \pm 4.3\%$. Bicuculline did not inhibit these responses to GABA.

4 Baclofen ($ED_{50} \pm$ s.e.mean, $6.8 \pm 1.4 \times 10^{-7}$ M) produced a similar inhibition ($47.4 \pm 3.2\%$ maximum) but 3-APS had no effect.

5 GABA (10^{-4} M) had no effect on the tone of isolated mesenteric or internal carotid arteries from the rabbit, whether or not the tone was increased with 5-HT. Similarly, GABA (10^{-4} M) did not produce any change in the responses to transmural stimulation in isolated mesenteric or internal carotid arteries.

6 These findings are consistent with the presence of two types of GABA receptor on the rabbit basilar artery.

Introduction

γ-Aminobutyric acid (GABA) has been found to dilate isolated cerebral, but not peripheral, arteries from the dog. This effect was dose-dependent and specifically blocked by the GABA antagonists, picrotoxin and bicuculline (Fujiwara, Muramatsu & Shibata, 1975; Edvinsson & Krause, 1979). These findings suggested the existence of postsynaptic cerebrovascular receptors for GABA.

Bowery & Hudson (1979) demonstrated that GABA depressed the evoked release of transmitter from sympathetic nerve terminals, by an action on a second type of GABA receptor. These receptors were insensitive to the antagonist bicuculline and to the agonist 3-aminoproprane sulphonic acid (3-APS), but were sensitive to baclofen. Measurements of effects on transmitter release and binding studies, have confirmed the presence of this second type of receptor in rat brain. Hill & Bowery (1981) have designated them $GABA_B$ to distinguish them from the conventional, bicuculline-sensitive $GABA_A$ receptors.

We have examined the possibility that either or both types of GABA receptor may exist on rabbit isolated basilar artery.

Methods

New Zealand White rabbits of either sex, weighing 2–3 kg, were anaesthetized with sodium pentobarbitone (35 mg/kg, i.v.) and killed by bleeding. The basilar artery was dissected out rapidly, and trimmed to a segment 7–8 mm long. This ring segment was threaded on two parallel stainless steel wires, one fixed, and one connected to a Statham Universal transducer UC3 to record changes in the tension developed transversely to the axis of the artery. The artery was suspended in a 20 ml bath of Krebs solution at 37°C, and the apparatus adjusted to give a resting tension of 500 mg. The composition of the Krebs solution was (mM): NaCl 118.4, KCl 4.74, $CaCl_2$ 2.5, KH_2PO_4 1.18, $MgSO_4$ 1.19, $NaHCO_3$ 25, glucose 11, gassed with 95% O_2 and 5% CO_2. For comparison, some experiments were conducted using internal carotid and mesenteric arteries prepared in the same manner.

Responses were recorded, either to drugs added to the bath, or to transmural stimulation (1 ms, 10 V, 10 Hz for 15 s) applied through a pair of electrodes fixed either side of the vessel. At the end of some experiments, tetrodotoxin was added to give a final concentration of 10^{-6} to 5×10^{-6} M. This blocked the

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responses to electrical stimulation, but left intact the responses to noradrenaline, confirming that only intramural nerves were stimulated with these parameters.

Direct effects on the muscle.

In preliminary tests, GABA was found to have no effect, or to produce a minimal relaxation when the artery was under resting tension. Therefore 5-hydroxytryptamine (5-HT) was added to the bath (3×10^{-8} M) to increase the tone. During this time cumulative dose-response curves were obtained with GABA (either alone or in the presence of bicuculline), (±) baclofen and 3-APS.

Indirect effects.

At least three control responses to transmural stimulation were obtained at 5–10 min intervals before adding drugs to the bath. The response to the third stimulation after addition of agonist, or agonist with antagonist, was measured and any change expressed as a percentage of the third control response.

Results

Direct effects of γ-aminobutyric acid, baclofen and 3-aminopropane sulphonic acid

Exposure of the arteries to 5-hydroxytryptamine 3×10^{-8} M produced an increase in tone of approximately 220 mg, which remained steady for more than an hour. Under those conditions, addition of GABA produced a relaxation in six out of eight preparations studied. The effect developed slowly over 2–3 min, was well maintained, dose-dependent and could be reversed on washing. The maximal response was a reduction of approximately 70% of the induced tone and the $ED_{50} \pm$ s.e.mean was $2.4 \pm 1.1 \times 10^{-5}$ M (Figure 1a and Table 1).

Baclofen in concentrations up to 10^{-4} M was inactive in this preparation but 3-APS produced a maximal reduction of about 23% of the induced tone with an ED_{50} of $5.8 \pm 1.4 \times 10^{-5}$ M (Figure 1a and Table 1).

Bicuculline (3×10^{-7} and 3×10^{-6} M) alone did not alter the tone of the vessel, but it moved the GABA dose-response curve to the right (Figure 1b). However, the maximal response to GABA was also reduced suggesting that the antagonism may not have been competitive. Picrotoxin (10^{-7}–10^{-6} M) alone slightly increased the resting tension of the vessel as well as reducing the responses to GABA. These concentrations of picrotoxin also reduced the responses to transmural stimulation and to added noradrenaline (see below).

For comparison the responses of two peripheral vessels from the rabbit, the internal carotid ($n = 3$) and mesenteric ($n = 3$) arteries were examined for possible responses to GABA. In neither vessel did GABA (10^{-4} M) produce any effect either in the presence or absence of tone induced by 5-HT.

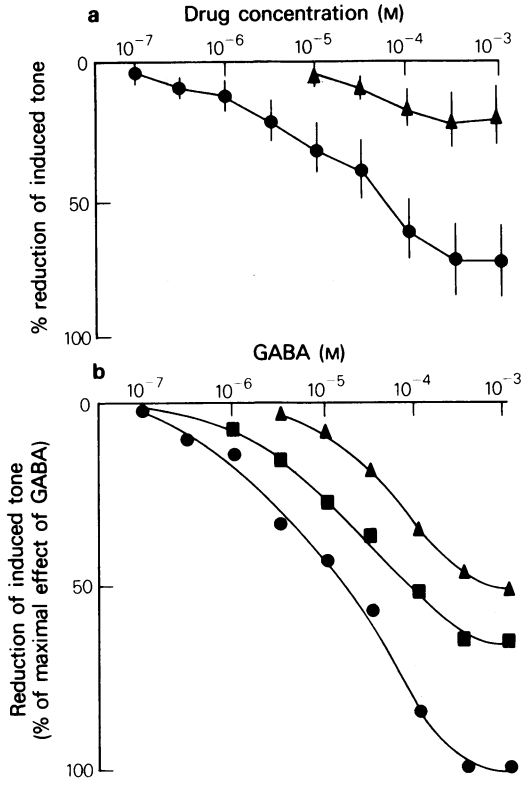

Figure 1 (a) Cumulative dose-response curves for the relaxant effects of γ-aminobutyric acid (GABA) (●) and 3-aminopropane sulphonic acid (3-APS) (▲) on isolated segments of the rabbit basilar artery previously contracted by 5-hydroxytryptamine (3×10^{-8} M). Points represent the means for 6 experiments with GABA and 4 experiments with 3-APS; vertical lines indicate s.e.mean. (b) The effect of bicuculline on the dose-response curve for the relaxant effects of GABA. The arteries were pretreated with bicuculline for 20 min: (●) no bicuculline; (■) 3×10^{-7} M; (▲) 3×10^{-6} M. Tone was then induced in the preparations by 5-hydroxytryptamine (3×10^{-8} M) and they were tested for responses to increasing concentrations of GABA. Each point represents the mean response for 3 experiments.

Table 1 Two actions of γ-aminobutyric acid (GABA) and related agonists on the responses of the rabbit isolated basilar artery

Drugs	*Direct vasodilator response*		*Inhibition of neurogenic constrictor responses*	
	Maximal dilatation (mg)	ED_{50} (M)	*Maximal inhibition* (% control)	IC_{50} (M)
γ-Aminobutyric acid	144 ± 21 $n=6$	$2.4 \pm 1.1 \times 10^{-5}$	49.2 ± 4.3 $n=9$	$5.6 \pm 2.1 \times 10^{-7}$
3-Aminoproprane sulphonic acid (3-APS)	43 ± 12 $n=4$	$5.8 \pm 1.4 = 10^{-5}$	0 $n=3$	$> 10^{-4}$
(±)-Baclofen	0 $n=3$	$> 10^{-4}$	47.4 ± 3.2 $n=4$	$6.8 \pm 2.4 \times 10^{-7}$

Values are mean ± s.e.mean.

Effects of γ-aminobutyric acid, baclofen and 3-aminoproprane sulphonic acid on the vasoconstrictor responses to transmural stimulation

GABA produced a dose-dependent inhibition of the constrictor responses of the isolated basilar artery to transmural stimulation in 9 out of 14 preparations studied. The range of effective concentrations was lower than that required for the direct response described previously and was 10^{-7}–5×10^{-6} M with an ED_{50} of $5.6 \pm 2.1 \times 10^{-7}$ M. The maximum inhibition was 49.2 ± 4.3% of the control responses (Figure 2a, Tables 1 and 2). Concentrations of GABA up to 10^{-6} M did not alter the responses to added noradrenaline (10^{-6} M) (Figure 2b). However, GABA in concentrations comparable to those producing a direct relaxation (5×10^{-6} M and above) did reduce the responses to noradrenaline (Figure 2b).

3-APS in concentrations up to 10^{-4} M ($n=3$) was inactive.

Baclofen was almost as effective as GABA in inhibiting the responses to transmural stimulation in 4 out of 6 preparations. The ED_{50} was $6.8 \pm 2.4 \times 10^{-7}$ M and the maximal inhibition was 47.4 ± 3.2% of the control response (Tables 1 and 2).

Bicuculline (10^{-6}–10^{-5} M) did not influence the inhibition of the responses to transmural stimulation produced by GABA ($n=6$) or baclofen ($n=3$) (Figure 2c). Picrotoxin 10^{-7}–10^{-6} M itself reduced the constrictor responses to transmural stimulation.

When peripheral vessels such as rabbit internal carotid ($n=6$) or mesenteric arteries ($n=4$) were used, GABA (10^{-4} M) did not produce any change in the responses to transmural stimulation or to added noradrenaline.

Discussion

These results suggest that two types of GABA receptor exist in the rabbit basilar artery. The action of GABA on the first group produces a relaxation of the vessel and they appear to be located postsynaptically. This is consistent with the observations of Fujiwara *et al.* (1975) and Edvinsson & Krause (1979) using cerebral vessels from the dog. These responses were only generated consistently with concentrations of GABA in excess of 10^{-6} M, but they could also be

Table 2 % inhibition by γ-aminobutyric acid (GABA) and related agonists of neurogenic constrictor responses of the rabbit isolated basilar artery

Drugs	*Concentration of drugs* (M) 10^{-7}	5×10^{-7}	10^{-6}	5×10^{-6}
GABA ($n=9$)	9.3 ± 3.2	22.5 ± 3.3	43.3 ± 2.8	49.2 ± 4.3
Baclofen ($n=4$)	6.4 ± 2.8	18.7 ± 4.6	39.2 ± 3.9	47.4 ± 3.2
3-APS ($n=3$)	0	0	0	0

Values are mean ± s.e.mean.
The magnitude of the effects of drugs is expressed in terms of percentage reduction of control responses.
3-APS = 3-aminopropane sulphonic acid.

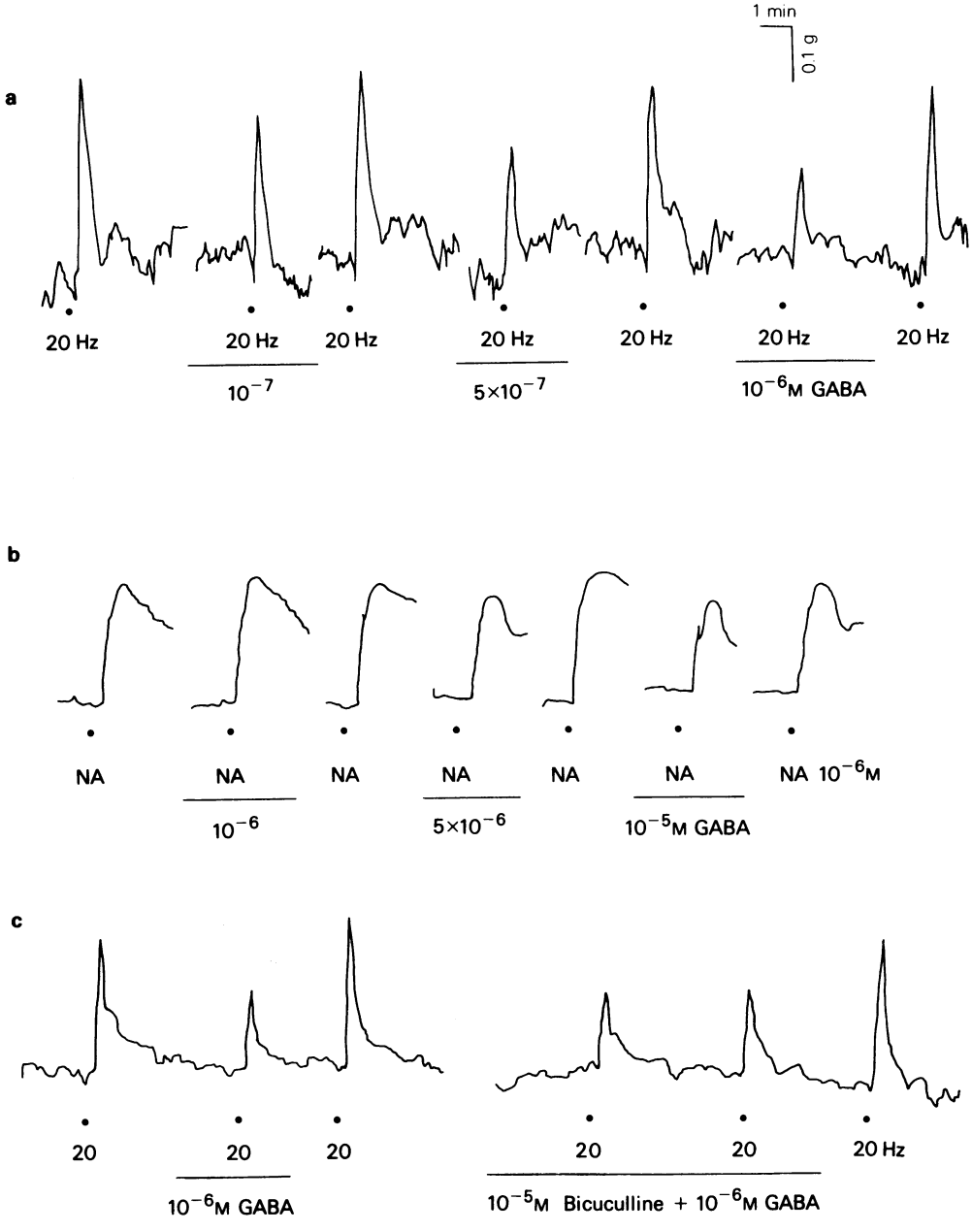

Figure 2 The effects of γ-aminobutyric acid (GABA) on the constrictor responses of the rabbit isolated basilar artery to transmural nerve stimulation and noradrenaline. (a) Increasing concentrations of GABA (10^{-7}–10^{-6} M) produced reversible and dose-dependent inhibition of the neurogenic constrictor responses. (b) GABA in a concentration of 10^{-6} M did not affect the constrictor responses to noradrenaline (added to the bath at the point (●) to give a concentration of 10^{-6} M). However, a dose of 10^{-5} M GABA did produce a small inhibition of the responses to noradrenaline. (c) Inhibition of the neurogenic constrictor responses by GABA (10^{-6} M) was not affected by preincubation of the tissue with bicuculline 10^{-5} M.

produced by 3-APS, and they could be antagonized by bicuculline, thus confirming their identity as 'classical' or $GABA_A$ receptors.

Picrotoxin produced an increase in tone of vessels and inhibition of the responses to GABA, noradrenaline and electrical stimulation. This lack of specificity made it unsuitable as a test for the receptor type.

Studies with internal carotid and mesenteric arteries failed to demonstrate the presence of these receptors in the peripheral vessels of the rabbit, again confirming the results of previous authors.

The second group of receptors appears to be located presynaptically since GABA reduces the responses to transmural stimulation in concentrations that have no effect on the responses to exogenous noradrenaline. They differ from the first group of receptors in several respects. Firstly, they are activated by lower concentrations of GABA (10^{-7}–10^{-6} M) than are required to activate the postsynaptic receptors (10^{-6}–5×10^{-4} M). Secondly, this effect is not produced by 3-APS but is produced by baclofen, the reverse of the situation seen with the postsynaptic receptors. Thirdly, these effects produced by GABA and baclofen are not inhibited by bicuculline. Therefore, they are not due to activation of the classical $GABA_A$ receptors, but appear to be mediated by a receptor mechanism similar to that designated $GABA_B$ by Hill & Bowery (1981). Since the inhibition of the response to transmural stimulation was produced with concentrations of GABA that did not modify the responses to added noradrenaline or to 5-HT, the effect is probably presynaptic rather than post-synaptic.

In our experiments using peripheral vessels no evidence could be obtained for a similar presynaptic receptor, but Starke & Weitzell (1980) have found evidence of inhibition of transmitter release and contractions by GABA using the pulmonary artery from the rabbit. The response only appeared at 10^{-6} M; and 10^{-3} M was required to produce a maximal effect, suggesting that the receptors involved were different. However, the response was resistant to blockade by either picrotoxin or bicuculline. The sharp differentiation in the distribution of these responses between the basilar artery and the peripheral arteries suggests that these receptors may have a physiological role in the control of the cerebral circulation. It is tempting to consider that the five preparations which did not respond to added GABA were already maximally affected by locally released GABA, but we have no evidence to offer in support of the hypothesis.

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