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$GABA_B$ receptor mediated inhibition of field stimulation-induced contractions of rabbit bladder muscle in-vitro

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The effects of GABA and selective $GABA_A$ and $GABA_B$ receptor agonists and antagonists have been investigated on field stimulation-induced contractions (0·1 Hz) of rabbit urinary bladder strips in-vitro. Atropine inhibits twitches of bladder strips obtained from the bladder dome more effectively than those obtained from the bladder base. Both GABA and the selective $GABA_B$ receptor agonist, (±)-baclofen inhibited field stimulation-induced contractions to about the same extent, while the selective $GABA_A$ receptor agonist, homotaurine had no effect. In the presence of atropine, GABA failed to inhibit further the amplitude of twitches. The effects of either GABA or (±)-baclofen were antagonized by the $GABA_B$ receptor antagonists homotaurine and 5-aminovaleric acid, while the $GABA_A$ receptor antagonist picrotoxin had no effect. Neither GABA nor (±)-baclofen had any significant effect on acetylcholine-induced contractions of unstimulated bladder strips, but they were abolished by atropine. These results suggest that $GABA_B$ receptors inhibit field stimulation-induced contractions of rabbit bladder muscle by reducing the amount of acetylcholine released per nerve impulse.

In recent years evidence has been provided that γ-aminobutyric acid (GABA) might play a modulatory role on the function of the peripheral nervous system (Jessen et al 1979; Krantis & Kerr 1981a; Taniyama et al 1982; Kerr & Krantis 1983).

Both facilitatory and inhibitory effects of GABA have been observed on acetylcholine release from cholinergic nerve endings of guinea-pig ileum (Krantis et al 1980; Bowery et al 1981; Krantis & Kerr 1981b; Kaplita et al 1982; Giotti et al 1983a, b; Kleinrock & Kilbinger 1983) and distal colon (Krantis et al 1980; Ong & Kerr 1983).

Two distinct types of GABA receptor appear to be involved in mediating the dual effects of this amino acid on the function of cholinergic neurons, namely $GABA_A$ and $GABA_B$ receptors (Bowery et al 1981) which can be differentiated by using of appropriate agonists and antagonists (Bowery et al 1981; Kaplita et al 1982; Giotti et al 1983).

Recently Taniyama et al (1983) described a bicuculline-sensitive (presumably mediated by $GABA_A$ receptors) inhibitory effect of GABA on the cholinergic component (atropine sensitive) of field stimulation-induced contraction of the guinea-pig urinary bladder in-vitro.

In this study we describe the effects of GABA and as well as those of selective $GABA_A$ and $GABA_B$ receptor agonists and antagonists on field stimulation-induced contractions of rabbit detrusor strips in-vitro.

Since in this animal species a gradient in sensitivity to cholinomimetics has been described (Van Buren & Anderson 1979; Levin et al 1980), we also investigated the effects of atropine and GABA on bladder muscle strips obtained from the bladder dome compared with the bladder base.

MATERIALS AND METHODS

Male albino rabbits, New-Zealand strain, 2·5–3·0 kg were killed by a blow on the back of the head and exsanguinated. The whole urinary bladder was rapidly removed and placed in an oxygenated (96% O_2 plus 4% CO_2) Krebs solution of the following composition (mM); NaCl 119, $NaHCO_3$ 25, KCl 4·7, $MgSO_4$ 1·5, KH_2PO_4 1·2, $CaCl_2$ 2·5 and glucose 11. Strips of bladder muscle were longitudinally cut from the bladder base (below the insertion of the ureters) or from the bladder dome (1 cm apart from the apex of the bladder). The strips were mounted in a 5 ml organ bath and suspended at a resting tension of 1 g from a isometric strain gauge connected to a Basile 7050 Unirecord. Temperature was maintained constant at 37 °C.

The preparations were field-stimulated by means of two platinum wire electrodes placed at the top and the bottom of the organ bath. Square wave pulses

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(60 V, 1 ms) were delivered automatically at 0·1 Hz by means of a Grass S 11 stimulator.

After a 30 min equilibration period the preparations were exposed to the various substances. A 30 min interval, with repetitive renewals of the bathing solution, was allowed to elapse between two administrations to avoid tachyphylaxis. Those preparations which received atropine, hexamethonium or tetrodotoxin (TTX) were challenged once only.

In a separate series of experiments the unstimulated preparations obtained from the bladder dome were challenged at 15 min intervals with acetylcholine (0·2 μM) until reproducible responses were obtained. The effects of GABA, (±)-baclofen or atropine on acetylcholine-induced contractions were assessed after a 5 min incubation period. At the end of the experiments the strips of bladder muscle were blotted and weighed; contractile values were expressed as mg mg^{-1} of wet weight.

All data are mean ± s.e. Statistical analysis was performed by means of the Student's *t*-test for paired or unpaired data. Drugs used were: γ-aminobutyric acid (GABA, Serva) (±)-baclofen (Ciba Geigy), homotaurine (3-aminopropansulphonic acid, Aldrich), picrotoxin (Serva), 5-aminovaleric acid (Janssen), atropine KCl (Serva), hexamethonium bromide (Serva), tetrodotoxin (TTX Sankyo).

RESULTS

Effect of atropine, phentolamine, hexamethonium and tetrodotoxin on field stimulation-induced contractions

Field stimulation (0·1 Hz, 60 V, 1 ms) of strips from the dome or the base of the urinary bladder produced isometric contractions whose amplitude was 26·1 ± 5 (n = 38) and 19·7 ± 4 (n = 36) mg per mg of wet weight, repectively. These values did not differ significantly. Atropine (3 μM) inhibited field stimulation-induced contractions of bladder strips from the dome more effectively than those from the base (36·4% and 16·7% inhibition, n = 12 and 11, respectively, $P < 0.01$, Fig. 1). Phentolamine (1 μM) and hexamethonium (10 μM) had no significant effect (n = 12). Tetrodotoxin (0·5 μM) produced over 90% inhibition of contractions in bladder strips from the dome, while a large tetrodotoxin resistant component (about 45%) was evident in bladder strips from the base; this could be attributed to a marked increase of spontaneous activity of the strips in presence of tetrodotoxin (cf Slack et al 1982).

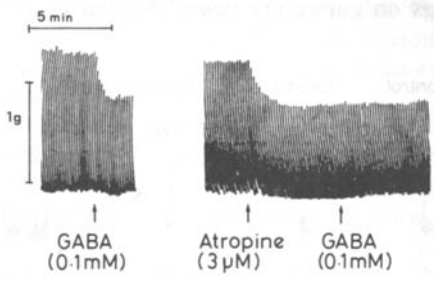

FIG. 1. Typical tracings showing the effect of GABA (0·1 mM) and atropine (3 μM) on field stimulation (0·1 Hz, 60 V, 1 ms)-induced contractions of rabbit urinary bladder smooth muscle. This experiment has been performed on a strip obtained from the bladder dome. Note that, in presence of atropine, GABA had no further inhibitory effect.

Effect of GABA, (±)-baclofen and homotaurine of field stimulation-induced contractions

The amplitude of field stimulation-induced contractions was reduced to the same extent by GABA (0·1 mM) and (±)-baclofen (0·1 mM) regardless of whether the strips had been obtained from the bladder dome (23·0 ± 4 and 21·2 ± 2% inhibition for GABA and (±)-baclofen respectively, n = 11, n.s. Fig. 1) or base (22·6 ± 3 and 22·4 ± 4% inhibition for GABA and (±)-baclofen respectively, n = 12, n.s.).

Homotaurine at 1 mM which was fully effective in stimulating $GABA_A$ receptors in the guinea-pig ileum (Giotti et al 1983a), had no effect on preparations from the bladder dome (n = 9). A slight but not significant inhibition (never exceeding 10%) was observed in 7 out of 8 preparations from the bladder base.

Effect of picrotoxin, homotaurine and 5-aminovaleric acid on GABA-induced inhibition of field stimulation-induced contractions

Previous experiments suggested that stimulation of the $GABA_B$ receptor subtype could be involved in GABA-induced inhibition of field stimulation-induced contractions of rabbit bladder dome or base.

Therefore we investigated the effects of picrotoxin, a selective $GABA_A$ receptor antagonist (Kaplita et al 1982; Kleinrok & Kilbinger 1983) and of both homotaurine and 5-aminovaleric acid, $GABA_B$ receptor antagonists (Muhyaddin et al 1982; Giotti et al 1983b), on the effects of either GABA or (±)-baclofen. The $GABA_A$ receptor antagonist, bicuculline, was not used because of its anticholinesterase activity (Svenneby & Roberts 1973). Neither picrotoxin (0·1 mM), homotaurine (1 mM) or 5-aminovaleric acid (2 mM) had any significant effect on the amplitude of the twitches. Exposure to homotaurine (1 mM, n = 10) or 5-aminovaleric acid (2 mM, n = 10) significantly reduced

the inhibitory effects of both GABA (0·1 mM, Fig. 2) and (±)-baclofen (0·1 mM), while picrotoxin, in a concentration (0·1 mM) that antagonized $GABA_A$ receptor mediated responses in guinea-pig ileum (Kaplita et al 1982), had no effect. Both homotaurine and 5-aminovaleric acid were slightly more active in reducing (±)-baclofen (72 ± 6 and 54 ± 4% inhibition respectively) than GABA (64 ± 6 and 46 ± 5% inhibition) effects on twitches.

Effect of atropine phentolamine and hexamethonium on GABA-induced inhibition of field stimulation-induced contractions

Previous exposure to atropine (3 μM) abolished almost completely the inhibitory effect of GABA (0·1 mM) on the amplitude of the twitches (Fig. 1) in preparations obtained either from the bladder dome or base (n = 5 and 4, respectively). On the other hand previous exposure to phentolamine (1 μM) or hexamethonium (10 μM) did not significantly reduce GABA (0·1 mM) effects on the amplitude of the twitches in bladder dome or base (n = 12).

Effect of GABA, (±)-baclofen and atropine on acetylcholine-induced contractions

Acetylcholine (0·2 μM) produced tonic contractions of bladder strips obtained from the bladder dome with a mean amplitude (22·9 ± 3·1 mg mg^{-1} of wet weight) not significantly different from that produced by field stimulation at 0·1 Hz.

Atropine (3 μM, n = 5) almost completely suppressed acetylcholine (0·2 μM)-induced contractions, while neither GABA (0·1 mM, n = 6) or (±)-baclofen (0·1 mM, n = 5) had any significant effect. A higher concentration of acetylcholine (200 μM) still elicited a slight contractile response in the presence of atropine (3 μM, Fig. 3).

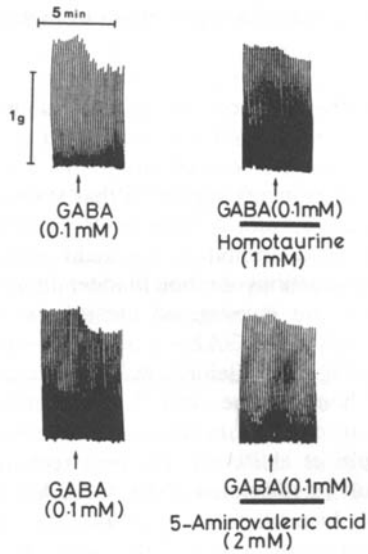

FIG. 2. Typical tracings showing the effects of homotaurine (1 mM upper panels) and 5-aminovaleric acid (2 mM, lower panels) on GABA-induced inhibition of field stimulation (0·1 Hz, 60 V, 1 ms)-induced contractions of rabbit bladder smooth muscle. Both strips were obtained from the bladder dome.

DISCUSSION

Our findings suggest that prejunctional $GABA_B$ receptors inhibit field stimulation-induced contractions in rabbit bladder smooth muscle by reducing the amount of acetylcholine released per nerve impulse. This conclusion stems from the following: (a) the selective $GABA_B$ receptor agonist (±)-baclofen (Bowery et al 1981) mimicked the effects of GABA, while homotaurine, a $GABA_A$ receptor agonist (Kaplita et al 1982; Giotti et al 1983) did not; (b) the $GABA_B$ receptor antagonists homotaurine (Giotti et al 1983) and 5-aminovaleric acid (Muhyaddin et al 1982) antagonized GABA and (±)-baclofen inhibition of twitches, while the $GABA_A$ receptor antagonist, picrotoxin (Kaplita et al 1982) did not; (c) the maximal inhibitory effect produced by GABA was not significantly different from that produced by (±)-baclofen in preparations from bladder dome or base; (d) GABA was not effective when the cholinergic component of twitches was removed by exposure to atropine and (e) neither GABA nor (±)-baclofen modified the effects of acetylcholine at the postjunctional level (cf. Maggi et al 1983). Therefore it could be hypothesized that in the rabbit urinary bladder $GABA_B$ receptors play a modulatory role on excitatory postganglionic neurotransmission by inhibiting acetylcholine release.

This interpretation agrees well with previous findings on guinea-pig ileum (Kaplita et al 1982;

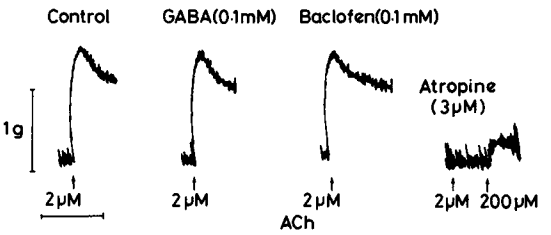

FIG. 3. Typical tracings showing the effects of GABA (0·1 mM) (±)-baclofen (0·1 mM) and atropine (3 μM) on acetylcholine (2 μM)-induced contraction on rabbit bladder smooth muscle. The strip had been obtained from the bladder dome.

Giotti et al 1983a, b; Kleinrock & Kilbinger 1983) and distal colon (Ong & Kerr 1983).

Noticeable species-related differences appear to occur in the modulatory role played by GABA on excitatory postganglionic innervation of the urinary bladder. In a previous paper we failed to observe any inhibitory effect of GABA on field stimulation induced contractions of rat urinary bladder at 1 Hz (Maggi et al 1983). Similar findings were obtained by using a lower frequency of stimulation (0·1 Hz) (unpublished observation) which has been routinely used in studies on guinea-pig ileum (Kaplita et al 1982; Kleinrock & Kilbinger 1983; Ong & Kerr 1983; Giotti et al 1983b). On the other hand Taniyama et al (1983) observed a bicuculline-sensitive, chloride ion-dependent (presumably mediated by $GABA_A$ receptors) effect of GABA on the cholinergic (atropine sensitive) component of field stimulation induced contractions of guinea-pig urinary bladder at 10 Hz.

In this study the inhibitory effect of GABA on the cholinergic component of the excitatory postganglionic neurotransmission of rabbit urinary bladder can be attributed to the activation of $GABA_B$ receptors. However it is unlikely that differences in responses to GABA between guinea-pig and rabbit urinary bladder are attributable to differences in frequency of stimulation. In fact, in preliminary experiments (±)-baclofen inhibited, while homotaurine had no effect on, contractions of rabbit bladder strips elicited at 10 Hz (unpublished observations).

Strong evidence exists indicating that the urinary bladder of both rabbit and guinea-pig receives both cholinergic and non-cholinergic, non-adrenergic excitatory postganglionic innervation (Burnstock et al 1972, 1978; Dean & Downie 1978). Therefore our observations are not at variance with those of Taniyama et al (1983) who reported that GABA effects on excitability of postganglionic neurons are confined to the cholinergic component of the excitatory innervation.

A gradient in sensitivity to cholinomimetics (Van Buren & Anderson 1979; Levine et al 1980) and muscarinic receptor density (Levine et al 1980, see also Anderson & Marks 1982; Johns 1983) has been described to occur when passing from the bladder base to the dome.

Further studies are needed to determine the physiological relevance of the gradient in cholinergic innervation in determining the intraluminal pressure increase during bladder voiding as well as the potential modulatory role of $GABA_A$ and $GABA_B$ agonists on these processes.

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