Table 1. Results of eight oxytocin bioassays using the mouse anococcygeus muscle.

Actual ratio of potency	Estimated ratio of potency	Fiducial limits (95%)	Index of precision (S/b; Holton 1948)
1·0	1·07	1·02–1·11	0·016
1·0	1·00	0·83–1·17	0·06
1·0	1·01	0·83–1·20	0·08
1·1	1·17	1·00–1·35	0·05
1·2	1·20	1·02–1·42	0·06
1·2	1·20	1·11–1·30	0·03
1·2	1·17	1·13–1·20	0·028
1·3	1·32	1·24–1·41	0·023

to obtain fiducial limits of the estimates of potency ratios. Over the eight assays, the mean ± standard error of the index of precision was 0·043 ± 0·008.

In some other experiments, the tissue was superfused with Krebs solution at a rate of 3·5 ml min^{-1}. Although such preparations gave dose-related contractions to oxytocin, the sensitivity was less than in the static organ bath.

In conclusion, the mouse anococcygeus muscle provides a useful tissue for the bioassay of synthetic oxytocin. Its sensitivity is comparable with that of the rat uterus (Follett & Bentley 1964) and the rat mammary myoepithelium (Polacek et al 1967); the stability of the mouse anococcygeus, and the fact that it is taken from male animals with no apparent dependence on hormonal state, suggests that it may have some advantages over the latter two preparations as an inexpensive bioassay of synthetic oxytocin. Further, the preparation has the advantage that a wide range of doses can be accommodated on the linear part of the dose-response curve. The relatively long dose-interval (8 min) means that two or more preparations can easily be managed concurrently.

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GABA inhibits excitatory neurotransmission in rat pelvic ganglia

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Intravenous GABA inhibited, in a bicuculline-sensitive manner, contractions of the urinary bladder induced by preganglionic nerve stimulation in urethane anaesthetized rats, while it had no significant effect on contractions produced by postganglionic nerve stimulation. In addition, intravenous GABA strongly inhibited DMPP-induced bladder contractions; this effect was also prevented by bicuculline. These experiments suggest that GABA inhibits excitatory neurotransmission in rat pelvic ganglia through a mechanism involving, at least in part, the activation of postsynaptic GABA A receptors.

In recent years much attention has been devoted to determine the potential effects of γ-aminobutyric acid (GABA) on the excitatory neurotransmission to the urinary bladder in various animal species (Sillen 1980; Maggi et al 1983, 1984; Taniyama et al 1983; Santicioli et al 1984; Kusunoki et al 1984).

We have previously reported that intravenous GABA, in a bicuculline-sensitive manner, transiently inhibits the micturition reflex in urethane anaesthetized

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rats (Maggi et al 1983). In adult rats, GABA appears to be devoid of significant effects on postganglionic excitatory neurotransmission (Maggi et al 1983, 1984). Since little intravenous GABA crosses the blood brain barrier (Kuriyama & Sze 1971), we hypothesized that, in our experimental conditions, GABA would impair neurotransmission at pelvic ganglia level (Maggi et al 1983). Interestingly, Taniyama et al (1983) described, in the guinea-pig isolated bladder, a bicuculline-sensitive inhibitory effect of GABA on the cholinergic component of the postganglionic excitatory neurotransmission. Unlike guinea-pigs, the rat bladder is almost devoid of intramural ganglion cells (Elmer 1978; Kusunoki et al 1984), so we thought that the same biological effect (i.e. a bicuculline-sensitive reduced excitability of postganglionic neurons) had been observed under different experimental conditions (in-vivo but not in-vitro in rats, in-vitro in guinea pigs), because of species-related anatomical differences (Maggi et al 1984).

Therefore, it appeared worthwhile to determine, in urethane anaesthetized rats, the potential effects of i.v. GABA on contractions produced by both pre- and postganglionic stimulation of pelvic nerves, as well as by chemical activation (DMPP) of postganglionic neurons.

Materials and methods

Male albino rats Wistar-Morini strain, 340–360 g, were anaesthetized with subcutaneous urethane ($1 \cdot 2$ g kg^{-1}) and the left jugular vein cannulated for drug injection. Through a midline incision of the abdomen the urinary bladder was exposed, emptied of urine by application of a slight manual pressure and prepared for recording of the intraluminal pressure as follows: through a small urethral incision a polyethylene tubing ($1 \cdot 0$ mm i.d., $1 \cdot 5$ mm o.d.) was inserted into the urinary bladder and secured by a silk ligature. The tubing was connected to a pressure transducer and the whole system filled with saline ($0 \cdot 9\%$ NaCl). Pressure signals were delivered to a H.P. 8805B carrier amplifier and displayed on a H.P. four channel polygraph. Cotton wool swabs soaked in warm saline were laid around the exteriorized organ to maintain its temperature and keep it moist.

Pre- or postganglionic stimulation of the excitatory nerve supply to the urinary bladder was affected in animals whose bladders were filled with saline in amounts ($0 \cdot 2$–$0 \cdot 3$ ml) insufficient to trigger reflex contractions. Preganglionic stimulation was as follows: the ventral sacral root S1 was isolated a few mm after emergence from the spinal cord and mounted on a bipolar platinum hook electrode. Postganglionic stimulation of the excitatory nerve supply to the bladder was at the level of the right ureter by means of bipolar platinum hook electrodes as described by Vanov (1965). In both types of experiments the abdomen was filled with mineral oil to avoid spread of current.

Square wave pulses were delivered by means of a Grass S11 stimulator at a frequency of 5 Hz (1 ms pulse width) with train of 5 s every 60 s. Voltage was 10–15 and 20–25 V for pre- and postganglionic stimulation, respectively.

In other experiments, after a 15 min equilibration, the ganglionic stimulant, DMPP was injected intravenously at 20 min intervals until two or more reproducible responses were obtained. This was done to evaluate the potential effects of GABA on the excitability of postganglionic neurons in the pelvic ganglia.

The effects of GABA on contractions produced by electrical stimulation of the excitatory nerve supply to the bladder was investigated either in control or bicuculline treated preparations.

Statistical analysis. All data in the text are mean ± s.e. Statistical analysis of the data was by means of the Student's t-test for paired or unpaired data when applicable.

Drugs. Drugs used were: GABA (Serva), atropine HCl (Serva), hexamethonium bromide (Serva), tetrodo-

toxin (TTX, Sankyo), dimethylphenylpiperazinium iodide (DMPP, Fluka), bicuculline (Serva).

Results

Electrical stimulation (5 Hz) of ventral sacral roots (S1) produced reproducible phasic contractions (19 ± 3 mm Hg, n = 18) which were marked (89 ± 2%) and prolonged (over 15 min); inhibition by intravenous hexamethonium (20 mg kg^{-1}, n = 4, Fig. 1C) confirmed their preganglionic origin. Atropine ($0 \cdot 2$ mg kg^{-1}, n = 4) had a slight (about 15%) inhibitory effect. Intravenous GABA in a dose (10 mg kg^{-1}, n = 5) shown previously to produce a transient inhibition of micturition reflex in urethane anaesthetized rats (Maggi et al 1983) reduced amplitude of these contractions (33 ± 3% inhibition, n = 5) (Fig. 1A). The effect of GABA was transient and amplitude of contractions recovered within a few min (Fig. 1A).

In preparations receiving i.v. bicuculline ($0 \cdot 3$ mg kg^{-1} 3 min before, n = 5), the effects of GABA were about halved ($P<0 \cdot 05$) compared with controls. In 2 out of 5 preparations a transient phasic contraction was observed just after bicuculline injection.
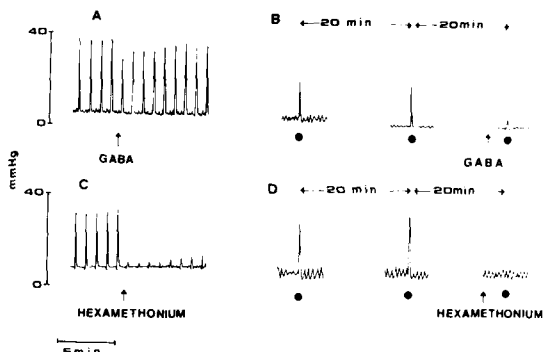

Fig. 1. Panel A. Effect of GABA (10 mg kg^{-1} i.v., at the arrow) on contractions of the rat urinary bladder produced by electrical stimulation (5 Hz, 5 s every 60 s) of ventral sacral roots. Panel B. Effect of GABA (10 mg kg^{-1} i.v. at the arrow) on contraction of the rat urinary bladder induced by i.v. DMPP (50 μg kg^{-1}, at the dots). Panel C. Effect of hexamethonium (20 mg kg^{-1} i.v., at the arrow) on contractions of the rat urinary bladder produced by electrical stimulation (5 Hz, 5 s every 60 s) of ventral sacral roots. Panel D. Effect of hexamethonium (20 mg kg^{-1} i.v., at the arrow) on contractions of the rat urinary bladder induced by i.v. DMPP (50 μg kg^{-1}, at the dots).

Electrical stimulation (5 Hz) of the right ureter produced reproducible phasic contractions whose amplitude was 18 ± 3 mmHg (n = 13). Hexamethonium (20 mg kg^{-1} i.v.) had no effect, thus indicating their postganglionic origin. Atropine ($0 \cdot 2$ mg kg^{-1}) had a slight (about 15%) inhibitory effect, while GABA (10 mg kg^{-1}) had no effect in 3 out of 5 preparations and induced a slight enhancement of contractions in the remainder.

Intravenous DMPP (50 µg kg⁻¹) at 20 min intervals induced reproducible phasic contractions of the rat bladder with a mean amplitude of 14 ± 2 mmHg (n = 13). These were abolished by intravenous hexamethonium (20 mg kg⁻¹, n = 4, Fig. 1E) while GABA (10 mg kg⁻¹, n = 5, Fig. 1B) produced a $78 \pm 6\%$ inhibition. In preparations receiving i.v. bicuculline (0·3 mg kg⁻¹, n = 4) the inhibitory effects of GABA were markedly reduced (about 85% inhibition compared with controls, $P < 0.02$).

Discussion

Our findings provide evidence indicating that GABA inhibits the excitatory neurotransmission in rat pelvic ganglia. The effect of GABA could be mediated, at least in part, by postsynaptic GABA A receptors as suggested by the bicuculline-sensitive GABA inhibition of DMPP-induced contractions. However, bicuculline was less effective in antagonizing GABA inhibition of contractions induced by preganglionic nerve stimulation. Therefore we cannot exclude that GABA inhibition of contractions induced by preganglionic nerve stimulation is due, at least in part, to the activation of presynaptic GABA B receptors.

Our observations are in keeping with previous findings which similarly indicate that GABA inhibits neurotransmission in dog (Stanton 1963) and cat (De Groat 1970) pelvic ganglia. The effect of GABA on contractions induced by preganglionic stimulation was modest (compared with hexamethonium) and transient. Since the same dose of GABA produces cardiovascular changes which last for 5 to 10 min (Giuliani, unpublished data), the transient nature of GABA action is likely to be due to some peculiarities in its behaviour at pelvic ganglia level.

Transient responses to GABA were also observed in isolated ganglionic preparations and, apart from desensitization of GABA receptors, might be due to a high affinity transport into glial cells similar to that described in the rat superior cervical ganglion (Bowery & Brown 1974) and cat dorsal root ganglion (Gallagher et al 1983).

The present findings with GABA parallel those with clonidine described by Walland (1984a, b) who found that, in the cat stellate ganglion, clonidine suppressed DMPP-induced responses but was barely effective toward responses produced by preganglionic nerve stimulation. This suggests that preganglionic nerve stimulation produces a supramaximal nicotinic activation in the ganglion which overcomes the inhibition produced by substances like clonidine (or GABA), which reduce excitability of postganglionic ganglion cells (as indicated by their effectiveness in antagonizing the effects of DMPP) (cf. also Walland 1984b).

Our present data confirm previous findings (Maggi et al 1983; 1984) indicating that GABA barely affects contractions of the rat bladder induced by postganglionic nerve stimulation.

Kusunoki et al (1984) demonstrated that GABA can be released from intramural neurons in the guinea-pig urinary bladder and proposed that, in this species, GABA acts as an inhibitory neurotransmitter in modulating acetylcholine release from postganglionic cholinergic neurons. Our findings indicate that, in rats, GABA modulates excitatory neurotransmission at pelvic ganglia through a mechanism (bicuculline-sensitive) which might be similar to that described in the guinea-pig isolated bladder.

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