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Intracisternal glycine activates the micturition reflex in urethane-anaesthetized rats

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Intracisternal glycine, but not GABA, activates a series of neurogenic rhythmic contractions of the urinary bladder in urethane-anaesthetized rats. This effect of glycine was prevented by strychnine but not by bicuculline, indicating the involvement of specific glycinergic receptors. The effects of glycine were also prevented by either atropine or haloperidol suggesting an involvement of cholinergic and monoaminergic excitatory neurotransmission to the bladder.

Glycine, one of the major inhibitory neurotransmitters in the central nervous system, is widely distributed in the spinal cord where it serves as transmitter for presynaptic inhibition (Snyder 1975). Exogenous glycine inhibits firing of sacral parasympathetic neurons (De Groat, 1970). It has been proposed that endogenous glycine participates in the physiological regulation of the micturition reflex by suppressing the reflexly activated firing of preganglionic neurons in the sacral spinal cord (De Groat and Ryall 1968).

In the course of experiments aiming to investigate the effects of i.v. glycine on the micturition reflex in urethane-anaesthetized rats we observed that this amino acid, depending upon the dose and/or the degree of activation of the bladder, produces either excitatory or inhibitory effects on bladder motility.

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In normal rats, as in cats and man, activation of a pontine centre induces micturition (Sato et al 1975, 1983; De Groat 1975; Satoh et al 1978). High levels of glycine (Aprison et al 1969) as well as high affinity uptake systems (Bennett et al 1973) and specific strychnine binding sites (Young & Snyder 1973) are present in the medulla oblongata-pons suggesting that glycine might serve as a neurotransmitter at this level (Snyder 1975).

Therefore it appeared worthwhile to determine whether the excitatory effects observed in some animals following i.v. glycine could be attributed to an effect on supraspinal centres involved in the regulation of the micturition reflex.

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 was cannulated for drug injection. Urethane was chosen as anaesthetic because it was shown to allow the study of both excitatory and inhibitory mechanism(s) on the micturition reflex (Maggi et al 1983, 1984a, b). The urinary bladder was exposed through a midline incision of the abdomen, emptied of urine by slight manual pressure, cannulated with a polyethylene tubing, and prepared for recording

of intraluminal pressure variations to infusions of saline (cystometrogram) (Maggi & Meli 1982a, b, 1983). The tip of the tubing was provided with two openings to minimize the potential interference of tissue occlusion on pressure recordings. The tubing was connected to an H.P. 1290 pressure transducer and the whole system filled with 0·9% NaCl (saline). Intraluminal pressure signals were delivered to an H.P. 8805B carrier amplifier and displayed on a H.P. 7754A four channel polygraph. Warm saline-soaked cotton wool swabs were laid around the exteriorized organ to maintain its temperature and keep it moist.

In some experiments spinal rats were obtained by severing the cord at the level of the intervertebral space T12-L1 under ether anaesthesia. The skin was closed with wound clips and the animals allowed to recover for 3–4 h before the administration of s.c. urethane.

After a 15 min equilibration period at zero volume the bladders were rapidly filled with 0·5–1·0 ml saline. This manoeuvre produced a series of distension-induced rhythmic contractions (DIRCs). In some experiments when DIRCs had reached steady values in frequency and amplitude a small amount of fluid (0·2 ml at each time) was withdrawn from the bladder until DIRCs disappeared, i.e. the bladder was brought to a subthreshold volume. At this time the bladder was allowed to equilibrate for a further 5–10 min before drug administration.

In some experiments the animals were placed on a stereotaxic apparatus and a midsagittal incision was made in the nuchal area; a 26 gauge needle connected to a syringe was placed at 2·0–2·5 mm midcaudally to the squama occipitalis. The needle was inserted downward and a slight reduction in pressure was applied to the syringe till appearance of cerebrospinal fluid. Glycine or GABA were administered in a volume of 20 µl.

In other experiments we attempted to establish the pattern of pressure changes occurring in rats during micturition and to compare them with characteristics of DIRCs. In these experiments the bladder was filled with saline by means of a 20 gauge needle inserted into the bladder dome. The needle was connected to a double lumen catheter which allowed simultaneous recording of intravesical pressure and saline infusion (which was made, at a rate of 0·175 ml min^{-1}, by means of a De Saga 131900 peristaltic pump). In these conditions the bladder was left free to void and saline infusion continued until micturition.

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

Drugs used were: glycine (Merck), tetrodotoxin (TTX, Sankyo), hexamethonium bromide (Serva), atropine HCl (Serva), GABA (Serva), strychnine nitrate (Sandoz), bicuculline HCl (Fluka), haloperidol (Serenase, Lusofarmaco).

Results

Rapid distension with 0·5–1·0 ml of saline produced a series of rhythmic contractions of the urinary bladder whose characteristics were 1·3 ± 0·3 contractions min^{-1} in frequency and 26 ± 2 mmHg in amplitude (n = 12). These contractions were abolished by topical TTX (10 µg in 0·1 ml, n = 6) or transiently suppressed by

Table 1. Effect of intracisternal glycine or GABA on bladder motility in urethane-anaesthetized rats.

Test Substance	Dose (µg)	Route	No.	Characteristics of DIRCs: Frequency (contractions min^{-1})	Characteristics of DIRCs: Amplitude (mm Hg)	No. of activated bladders	Characteristics of drug induced contractions: Frequency (contractions min^{-1})	Characteristics of drug induced contractions: Amplitude (mm Hg)
Controls (saline)	—	i.c.	12	1·3 ± 0·3	26 ± 2	1/12	—	—
Glycine	300	i.c.	15	1·2 ± 0·2	27 ± 2	13/15	1·2 ± 0·3	27 ± 2
GABA	500	i.c.	6	1·4 ± 0·2	25 ± 2	1/6	—	—
Glycine plus strychnine	300 500	i.c. i.v.	6	1·5 ± 0·1	26 ± 3	1/6	—	—
Glycine plus bicuculline	300 300	i.c. i.v.	7	1·3 ± 0·2	23 ± 3	5/7	1·3 ± 0·3	29 ± 4
Glycine plus haloperidol	300 200	i.c. i.v.	8	1·1 ± 0·4	30 ± 3	1/8	—	—
Glycine plus atropine	300 2000	i.c. i.v.	7	1·4 ± 0·4	21 ± 1	1/7	—	—

DIRCs are induced by rapidly filling the bladder with 0·5–1·0 ml of saline; when DIRCs reached a steady state a volume of 0·2–0·4 ml saline was withdrawn to bring the bladder to a subthreshold condition. After a further equilibration period either saline (controls) glycine or GABA were injected intracisternally in a volume of 20 µl.

Strychnine, bicuculline, haloperidol or atropine (doses are in µg kg^{-1}) were intravenously injected 3 min before i.c. glycine.

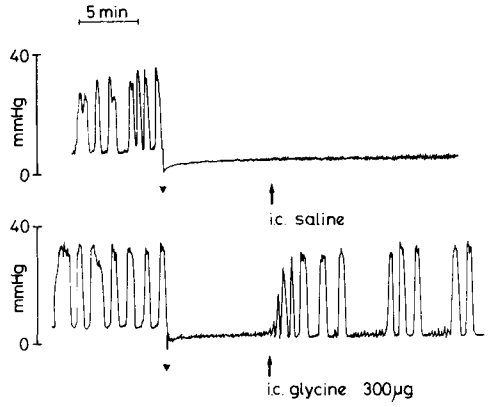

FIG. 1. Upper panel: Typical tracing showing the effect of saline withdrawal on the distension-induced rhythmic contractions (DIRCs) of the urinary bladder in urethane-anaesthetized rats. At the triangle, saline 0·2 ml was rapidly withdrawn. This produced disappearance of DIRCs. At the arrow i.c. saline (20 μl) was administered. Lower panel: Typical tracing showing the activatory effect of i.c. glycine (300 μg in 20 μl, at the arrow) on a bladder brought to a subthreshold condition by saline withdrawal (0·2 ml, at the triangle).

either i.v. atropine or hexamethonium (cf. Maggi et al 1983, 1984a, b). In acute spinal rats (T12-L1) rapid distension of the urinary bladder failed to produce sustained regular rhythmic contractions (n = 6).

Transvesical infusion of saline (0·175 ml min^{-1}) elicited micturition at a volume of 0·63 ± 0·08 ml (n = 16). Micturition was characterized (in these experimental conditions) by the stream-like emission of several drops of fluid during a sustained increase in intravesical pressure. The amplitude of the micturition contraction was 25 ± 1 mmHg which is not significantly different from that of DIRCs. Only in 2 out of 16 preparations micturition was produced at an intraluminal pressure lower than 20 mmHg. These experiments indicate that DIRCS most likely represent a repetitive micturition reflex of supraspinal origin whose rhythmic feature depends upon the occlusive ligature at urethral level which, by impeding the bladder voiding, prevents the stretching stimulus from being removed.

Removal of saline produced disappearance of DIRCs, indicating that bladder distension is required for their maintenance. DIRCs disappeared usually after the removal of 0·2–0·4 ml saline and did not reappear spontaneously for at least 20 min (n = 5). Intracisternal saline (5–10 min after bladder deflation) had no effect on bladder motility (n = 10, Fig. 1). Therefore such an experimental situation is suitable for testing the activating effect of substances on the supraspinal mechanism(s) regulating the micturition reflex.

Intracisternal glycine (300 μg) produced reappearance of DIRCs within 2 min in 13 out of 15 preparations (Fig. 1). Higher doses of glycine (500–1000 μg) produced respiratory failure, while a lower dose (100 μg) had no effect on bladder motility. Glycine-induced rhythmic contractions usually lasted for at least 10 min (range 5–20 min). Characteristics of frequency and amplitude as well as morphology of glycine-induced rhythmic contractions were similar to those of DIRCs (Table 1). In similar experimental conditions GABA (500 μg, n = 6) had no significant effect on bladder motility.

Intravenous strychnine (500 μg kg^{-1}, n = 6) prevented the activatory effects of i.c. glycine (300 μg) in 5 out of 6 preparations, while intravenous bicuculline (300 μg kg^{-1}, n = 7) did not prevent glycine-induced contractions. The i.v. injection of strychnine or bicuculline produced 1–3 phasic contractions of the bladder which lasted for 1–2 min in 1 out of 6 and in 2 out of 6 preparations, respectively.

It has been previously shown that both cholinergic and catecholaminergic mechanisms participate in the activation of the micturition reflex at CNS level (Sillen 1980). To test the hypothesis that such a mechanism was involved in our experimental conditions we investigated the effects of i.c. glycine (300 μg) in atropine (2 mg kg^{-1} i.v., 3 min before) or haloperidol (0·2 mg kg^{-1} i.v., 3 min before) treated animals. Both atropine and haloperidol pretreatment prevented glycine-induced bladder rhythmic contractions.

Discussion

Our findings indicate that in urethane-anaesthetized rats the stimulation of glycinergic receptors in the CNS activates a supraspinal micturition reflex presumably similar to that which, in this species, subserves bladder voiding.

Specificity of the glycinergic mechanism(s) operating in our experimental conditions is confirmed by the observation that: (a) GABA did not mimic the effects of glycine and (b) strychnine but not bicuculline prevented glycine-induced activation.

In barbiturate-anaesthetized rats Sillen et al (1980) reported that intracerebroventricular glycine suppressed L-dopa-activated bladder rhythmic contractions. Failure to observe excitatory effects of glycine by Sillen et al (1980) might be ascribed to a variety of factors such as differences in the site of injection, type of anaesthesia or the fact that, in our study, the bladders were brought to a subthreshold volume.

Although only limited pharmacological studies are available concerning the neurotransmitter(s) systems regulating the micturition reflex at CNS level, evidence is available that both cholinergic and monoaminergic systems are involved (Sillen et al 1980; Sillen 1980). In addition the postganglionic excitatory innervation to the rat bladder is, at least in part, cholinergic (Maggi et al 1983, 1984b). The observation that both atropine and haloperidol prevented the glycine-induced activation of bladder motility suggests that the glycine-induced rhythmic contractions of the rat bladder are produced (directly or indirectly) by activation of the same neural pathways which are involved in the genesis of the

supraspinal micturition reflex, although no conclusion on the site of action of these substances could be drawn from present findings. Such a hypothesis is also supported by the observation that glycine produces, through a specific, strychnine-sensitive mechanism, a release of dopamine (Giorguieff-Chesselet et al 1979; Kerwin & Pycock 1979) and acetylcholine (Beani et al 1983) at CNS level.

We cannot exclude that glycine-induced activation of micturition is indirect in nature, i.e. depends upon a facilitatory effect (perhaps due to disinhibition) on mechanism(s) regulating the pressure threshold for micturition. Alternatively we could hypothesize that glycine produces a direct activation of neuronal pathways which ultimately produce firing of preganglionic neurons in the sacral parasympathetic micturition centre. Further studies are needed to elucidate the mechanism(s) underlying the glycine-induced activation of micturition in urethane-anaesthetized rats as well as the potential role of glycinergic mechanism(s) in regulating the function of the vesicourethral complex.

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