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Selective inhibition by glycine of some somatic reflexes in the cat

B. N. DHAWAN, J. N. SHARMA AND R. C. SRIMAL

Division of Pharmacology, Central Drug Research Institute, Lucknow-1, India

Summary

1. The effects of intracerebroventricular or intrathecal administration of glycine on some somatic reflexes in chloralosed cats were investigated.

2. Glycine produced marked inhibition of the flexor reflex (FR) and crossed extensor reflex (CER) at low doses (2·5–5·0 mg) but even large doses (up to 40·0 mg) had no effect on the facilitation or inhibition of the patellar reflex produced by stimulation of either the reticular formation or the sciatic nerve. The linguo-mandibular reflex (LMR) could be blocked by 10–20 mg glycine.

3. Glycine antagonized strychnine-induced facilitation of FR, CER and LMR and was 5–6 times as active as γ-aminobutyric acid in this respect.

4. The possibility of glycine being a transmitter in spinal pathways is discussed.

Introduction

It has recently been suggested that amino-acids may play an important part in the normal function of the central nervous system and may also be transmitters at certain spinal synapses (Phillis, 1970). Aprison & Werman (1965) demonstrated the presence of relatively high amounts of glycine in the grey matter of the ventral and dorsal horns of the spinal cord, and very small amounts in the ventral root fibres. Davidoff, Shank, Graham, Aprison & Werman (1967) reported that glycine was located in the interneurones. Curtis, Hösli & Johnston (1968a) found that iontophoretically applied glycine produced marked inhibition in the rate of firing of spontaneously active spinal neurones. Further, the conductance changes in the postsynaptic nerve membrane were similar to those observed on the stimulation of presynaptic nerves. In addition, glycine antagonized the effects of strychnine on the postsynaptic nerve endings. On the basis of these observations they suggested that glycine could be the neurotransmitter at the inhibitory synapses in cat spinal cord. In view of these reports it was considered worthwhile to investigate the effects of glycine on some of the neuronal pathways which integrate the somatic reflexes; the results are reported here. A preliminary report of part of this work has been published elsewhere (Sharma, Gupta & Dhawan, 1971).

Methods

Cats weighing 3–4 kg were anaesthetized with chloralose (80·0 mg/kg i.v.). Glycine was administered either in a lateral cerebral ventricle (i.c.v.) through a Collison's cannula (Feldberg & Sherwood, 1954) or intrathecally (i.t.) by a hypodermic needle inserted in the third or fourth lumbar space. This was done to avoid

peripheral effects and to ensure adequate local concentration. The drug concentration was so arranged that the total volume injected at a time did not exceed 0·5 ml. The pH of the solution was between 6 and 7. Control administration of an equal volume of saline at this pH had no effect on the reflexes investigated. In some experiments an aqueous solution of γ-aminobutyric acid (GABA) was also similarly administered. The pH was the same as that of glycine. The blood pressure was recorded from a cannulated carotid artery either through a mercury manometer or through a Statham p23 dc transducer (1 mmHg≡1·333 mbar).

The effect on the following reflexes was examined:

Linguomandibular reflex (LMR). LMR was elicited by stimulating the root of the tongue by two needle electrodes (1–3 V, 3–6 ms at 12/min), while the movements of the lower jaw were recorded (King & Unna, 1954).

Crossed extensor reflex (CER). CER was elicited by stimulating the cut central end of the sciatic nerve (0·5–2 V, 2–4 ms at 10/min) and recording the extensor movements of the other leg.

Flexor reflex (FR). The cut central end of the medial branch of sciatic nerve was stimulated (1–4 V, 4–8 ms at 12/min) and the contractions of the tibialis anterior muscle were recorded.

Patellar reflex (PR) and its polysynaptic facilitation. PR was elicited by tapping the common tendon of quadriceps muscle by an electromagnetic hammer every 5 s (Calma & Wright, 1947). Stimulation of the cut central end of the contralateral sciatic nerve (0·5–2 V, 2–4 ms at 100 Hz for 10 s) produced facilitation of the PR. Facilitation of PR was also obtained by stimulation of the mid-brain reticular formation (RF) through a concentric needle electrode placed stereotaxically according to parameters described by Henneman, Kaplan & Unna (1949).

Monosynaptic and polysynaptic inhibition of PR. Monosynaptic inhibition of PR was obtained by stimulation of the central end of the cut ipsilateral sciatic nerve (0·5–2 V, 0·5 ms at 120 Hz for 10 s) while the contralateral nerve was cut (Abdulian, Martin & Unna, 1960). Polysynaptic inhibition of PR was obtained by stimulating either the cut central end of the contralateral sciatic nerve or the inhibitory area of RF (Henneman *et al.*, 1949).

Facilitation of polysynaptic reflexes with strychnine. Polysynaptic reflexes were facilitated by intrathecal or intracerebroventricular administration of 10–20 μg strychnine sulphate.

In some experiments pressor responses were obtained by electrical stimulation (0·5–5 V, 1 ms at 100 Hz for 5 s) of medullary vasomotor locii using a stereotaxically placed concentric needle electrode.

The blood pressure and the reflexes were recorded either on a kymograph or on a Grass model 7 polygraph. Each experiment was repeated in at least three cats. The electrical stimuli were delivered either from a Grass S4 stimulator or an Aplab Electronic stimulator.

Results

Effect on blood pressure

Glycine had no significant effect on the blood pressure level after intracerebroventricular (Figs. 1, 2) or intrathecal administration (Figs. 3, 4 and 6). Nor had

it any effect on the pressor responses to stimulation of medullary vasomotor loci up to a dose of 10 mg (i.c.v.). Fifteen minutes after GABA (10 mg i.c.v.) the blood pressure was slightly lowered and the vasomotor responses to medullary stimulation were inhibited. Fig. 1 shows the results obtained with glycine and GABA in a typical experiment.

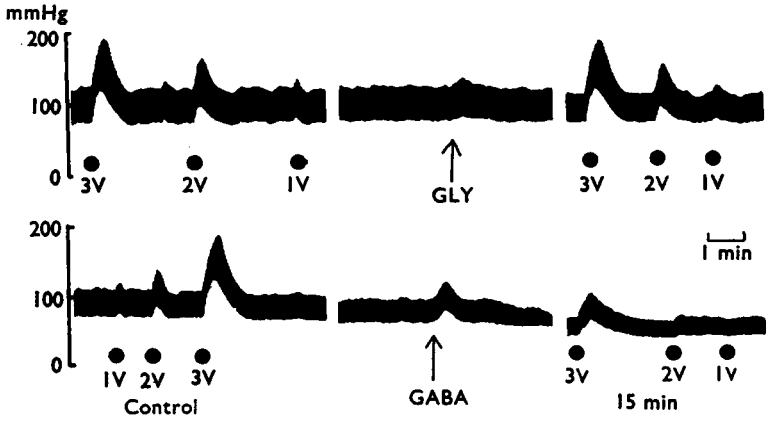

FIG. 1. Blood pressure record of a 3 kg anaesthetized (chloralose) cat. The medullary vasomotor centre was stimulated (100 Hz, 0·5 ms duration, 1–3 V for 5 s) through a bipolar concentric electrode at dots. The upper panel shows responses to 3 V, 2 V and 1 V stimulation. Glycine (GLY, 10 mg i.c.v.) produced no appreciable change either in the blood pressure or the vasomotor responses. The lower tracing shows the effect of gamma-aminobutyric acid (GABA, 10 mg i.c.v.). Note some lowering of the blood pressure, and blockade of the medullary responses to stimuli of 1 V and 2 V and inhibition of response to 3 V at 15 minutes.

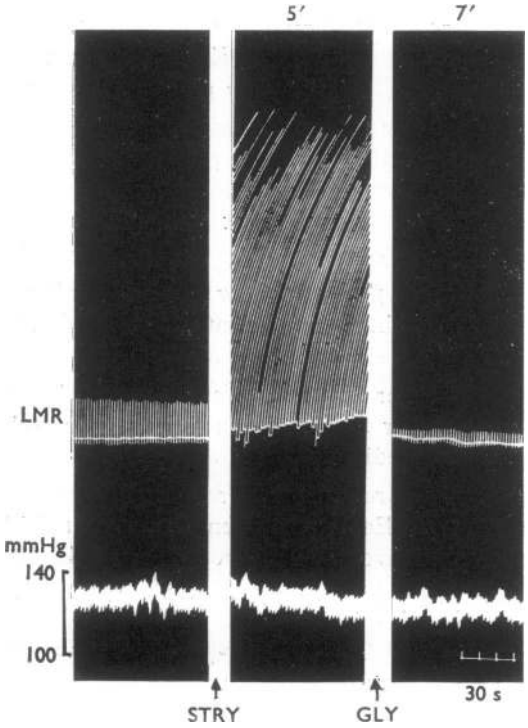

FIG. 2. Record of the linguomandibular reflex (LMR) and the blood pressure of a cat (2·8 kg). The LMR was facilitated by strychnine (STRY, 10 μg i.c.v.). The facilitated LMR was completely inhibited by glycine (GLY, 15·0 mg i.c.v.).

Effect on LMR

LMR was inhibited by 10–20 mg glycine (i.c.v.) but not by lower doses. Similar doses also antagonized the strychnine induced facilitation as shown in Fig. 2.

Effect on CER

CER was completely blocked for 15–20 min by glycine (2·5 to 5 mg i.t.). Strychnine (10 μg) induced facilitation of the reflex was also abolished by doses of 5–10 mg (Figs. 3 and 4).

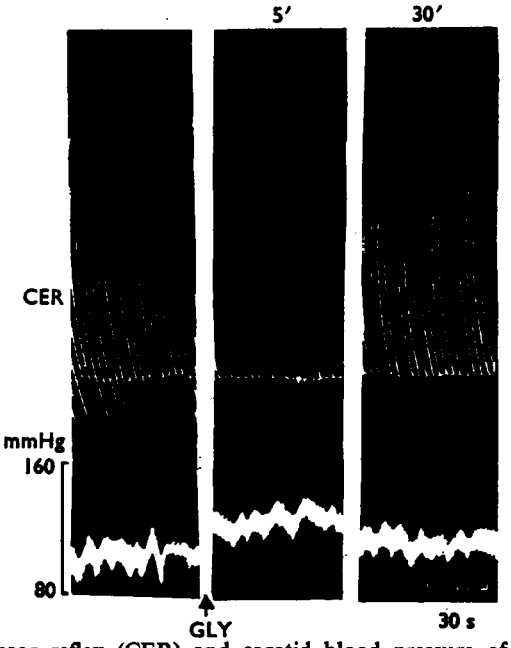

FIG. 3. Crossed extensor reflex (CER) and carotid blood pressure of an anaesthetized cat. Glycine (2·5 mg) was administered intrathecally (GLY). Note the complete block of CER (middle panel) and its partial recovery at 30 min (right panel).

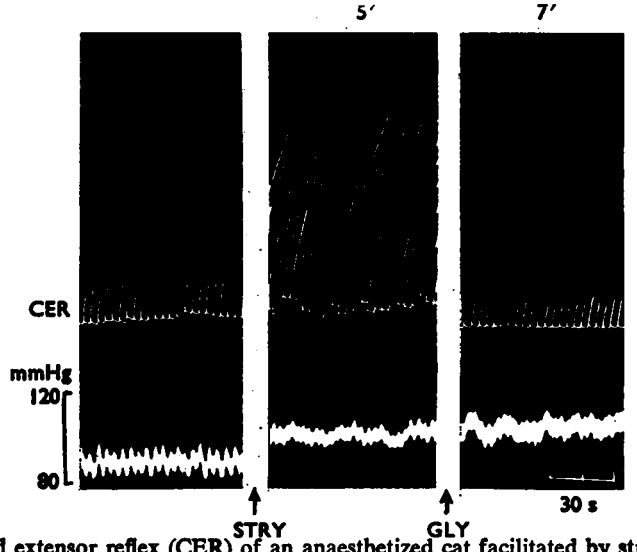

FIG. 4. Crossed extensor reflex (CER) of an anaesthetized cat facilitated by strychnine (STRY, 10 μg i.t.). The facilitated reflex (middle panel) was brought back to normal (right panel) by glycine (GLY, 5·0 mg i.t.).

Effect on FR

Relatively high doses of glycine (5–10 mg i.t.) were required to produce significant (80–100%) blockade of FR which lasted 30–50 minutes. A slightly higher dose of glycine (Fig. 5) was required to block strychnine-induced facilitation of FR. Much higher doses (30–50 mg) of GABA were required to produce an effect on FR.

Effect on other reflexes

Glycine, even at doses of 20–40 mg (i.c.v. or i.t.) failed to inhibit the PR or its facilitation and inhibition produced by stimulation of RF or sciatic nerve. (Figs. 6 and 7 respectively). In contrast to glycine, GABA (40 mg i.t.) partially blocked the inhibition of PR due to contralateral sciatic nerve stimulation (Fig. 7).

Discussion

Curtis & Watkins (1965) have summarized the evidence supporting the possibility of GABA acting as a neurotransmitter in the CNS. Several other naturally occurring amino-acids have recently been investigated for their effects on neuronal transmission (Phillis, 1970). Of these, most evidence has been presented in favour of

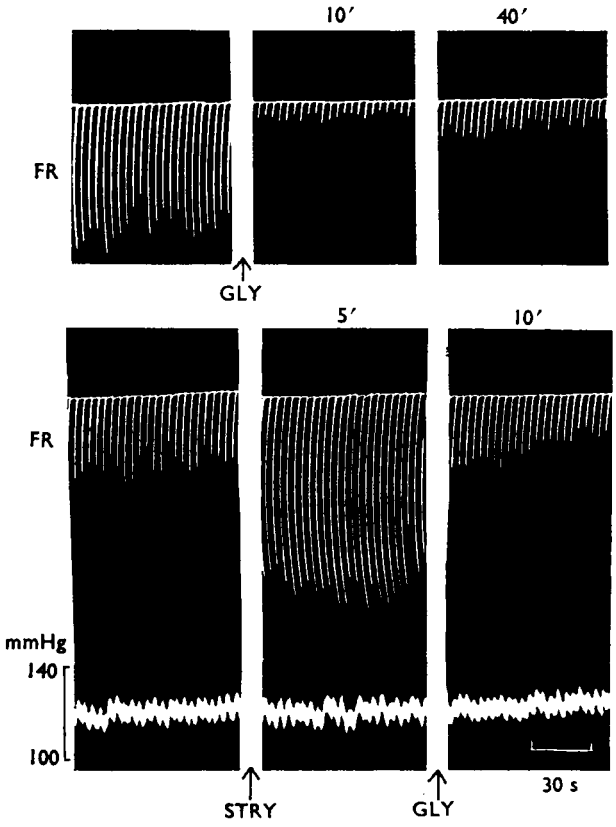

FIG. 5. Flexor reflex (FR) of an anaesthetized cat (3·2 kg). Upper tracing shows the effect of glycine (GLY, 5·0 mg i.t.) at 10 min (middle panel) and its partial recovery at 40 min (right panel). The lower tracing shows the FR (in the middle panel) facilitated by strychnine (STRY, 10 μg i.t.) and its antagonism by glycine (GLY, 7·0 mg i.t.) in the right panel.

glycine as a possible transmitter, particularly at the inhibitory spinal pathways (Curtis *et al.*, 1968a). The same authors have also demonstrated that when applied iontophoretically to spontaneously active (firing) neurones, glycine can effectively suppress the rate and amplitude of firing. They have also observed that glycine produces changes (hyperpolarization and increased ionic conductance to K^+ and Cl^-) in the postsynaptic nerve membrane which occur when the presynaptic nerve ending is stimulated. Glycine also effectively antagonizes the effects of strychnine on the postsynaptic nerve ending and the antagonism is competitive in nature (Curtis *et al.*, 1968a).

In the present series of experiments glycine had a marked inhibitory effect on the spinal somatic reflexes (CER and FR) at low doses (2·5–5 mg). It is much more potent than GABA (Bhargava & Srivastava, 1964) which depresses these reflexes in doses of 100–200 mg (i.t.). The marked depressant effect of glycine on the

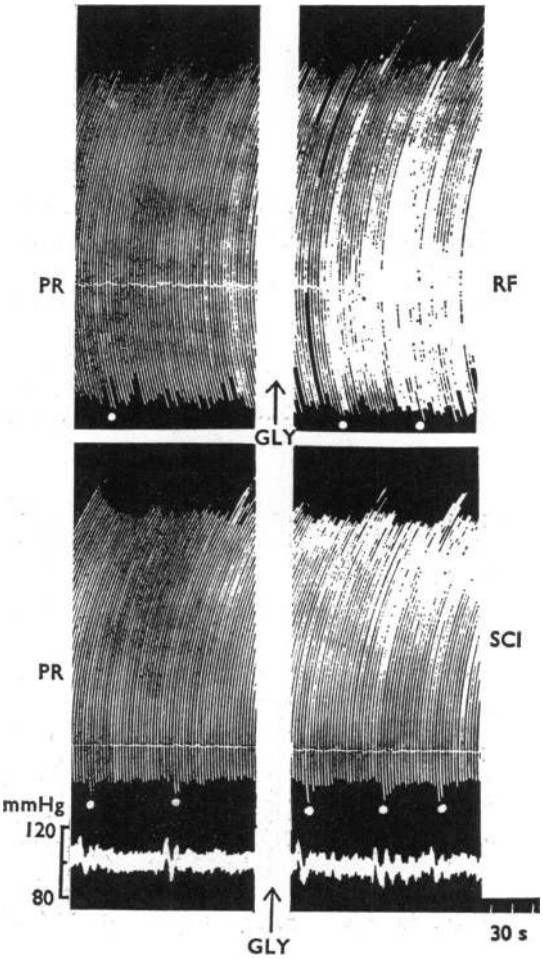

FIG. 6. Facilitation of the patellar reflex (PR) by stimulation of the facilitatory area of brain stem reticular formation (RF) is shown in the upper tracing. Note that glycine (GLY, 40·0 mg i.c.v.) failed to affect the facilitatory responses (dots). The lower tracing shows the facilitated responses due to contralateral sciatic stimulation (SCI) at dots. These responses were not inhibited by glycine (GLY, 40·0 mg i.t.). The blood pressure was unaffected.

reflexes appears to be due to its effect on the intermediate neurones of the spinal cord. A direct depressant effect on motor neurones appears less likely since glycine does not produce inhibition of all the reflexes uniformly. It does not inhibit the monosynaptic patellar reflex and its polysynaptic modification (inhibitory as well as facilitatory) by stimulation of reticular formation or the sciatic nerve. On the other hand, an inhibitory effect on these reflexes has been observed with GABA.

Glycine is much more potent than GABA in antagonizing strychnine-induced facilitation of somatic reflexes. Similar results have been reported by Curtis, Hösli, Johnston & Johnston (1968b) on spinal motoneurones. This further differentiates glycine from GABA although both are depressant amino-acids.

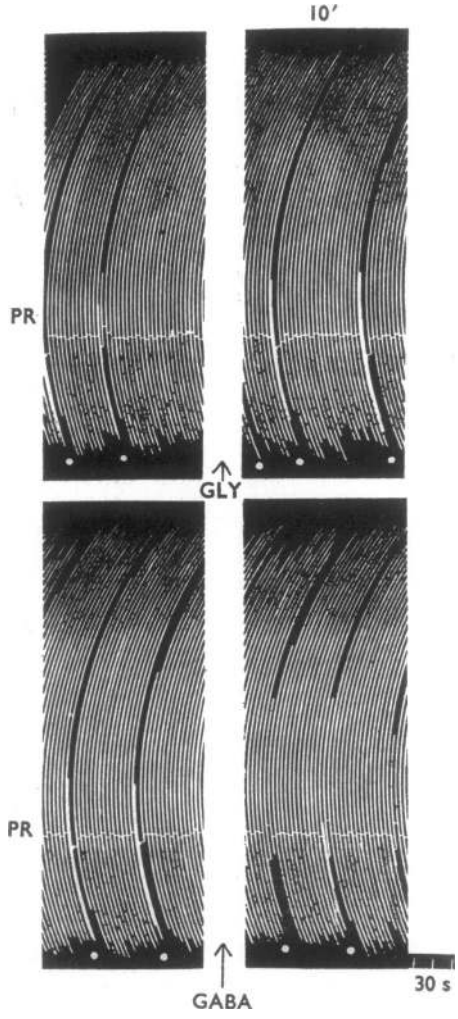

FIG. 7. Record of patellar reflex (PR) and its polysynaptic inhibition by contralateral sciatic stimulation (dots). Upper tracing shows the effect of glycine (GLY, 40·0 mg i.t.) and the lower tracing depicts the effect of gamma-aminobutyric acid (GABA, 40 mg i.t.) on its polysynaptic inhibition. Glycine had no effect on the PR and its inhibition while GABA partially blocked the inhibition of PR and also slightly reduced it at 10 minutes.

At the brainstem level, glycine did not block the facilitation and inhibition of PR due to stimulation of reticular formation nor did it inhibit the vasomotor locii. This action again contrasts with the effect of GABA which blocks all these responses uniformly. LMR is, however, inhibited by comparatively high doses of glycine and also by GABA.

Recently, Kelly & Krnjević (1969) investigated the effects of iontophoretically applied glycine on cortical neurones in cats. They have observed that glycine had only a transient depressant effect on the cortical neurones as compared to GABA and was only 25% as active in blocking the glutamate induced discharges. The failure of glycine in the present study to affect the reticular formation integration areas of somatic reflexes, also points to very weak or negligible effect of glycine on subcortical areas as well. Thus the main site of action of glycine appears to be the spinal cord where it may be acting as an inhibitory neurotransmitter at the strychnine sensitive sites.

It is evident from the results obtained in this investigation and from those reported in the literature that glycine is much more potent than GABA at the spinal cord level. The action of glycine is, in addition, more selective and only on some reflexes rather than a uniformly depressant action as is the case with GABA. Further, the association of glycine with the spinal interneurones (Davidoff *et al.*, 1967) and the demonstration of the release of ^{14}C-glycine from electrically stimulated rat spinal cord slices (Hopkin & Neal, 1970) are additional evidence in favour of glycine acting as an inhibitory neurotransmitter at this level. At the brainstem level glycine and GABA appear to be more or less equiactive, with the action of the former being more selective than that of the latter. At the cortical level GABA is definitely more active than glycine and the latter does not seem to act as a neurotransmitter.

REFERENCES

ABDULIAN, D. C., MARTIN, W. R. & UNNA, K. R. (1960). Effects of central nervous system depressants on inhibition and facilitation of the patellar reflex. *Archs int. Pharmacodyn. Thér.*, **128**, 169–186.

APRISON, M. H. & WERMAN, R. (1965). The distribution of glycine in cat spinal cord roots. *Life Sci.*, **4**, 2075–2083.

BHARGAVA, K. P. & SRIVASTAVA, R. K. (1964). Nonspecific depressant action of γ-aminobutyric acid on somatic reflexes. *Br. J. Pharmac. Chemother.*, **23**, 391–398.

CALMA, I. & WRIGHT, S. (1947). Effects of intrathecal injection of potassium chloride and other solutions in cats, and the excitatory action of potassium ions on posterior nerve root fibres. *J. Physiol., Lond.*, **106**, 211–235.

CURTIS, D. R., HÖSLI, L. & JOHNSTON, G. A. R. (1968a). A pharmacological study of the depression of spinal neurones by glycine and related amino acids. *Exp. Brain Res.*, **6**, 1–18.

CURTIS, D. R., HÖSLI, L., JOHNSTON, G. A. R. & JOHNSTON, I. H. (1968b). The hyperpolarization of spinal motoneurones by glycine and related amino acids. *Exp. Brain Res.*, **5**, 235–258.

CURTIS, D. R. & WATKINS, J. C. (1965). The pharmacology of amino acids related to gamma-aminobutyric acid. *Pharmac. Rev.*, **17**, 347–392.

DAVIDOFF, R. A., SHANK, R. P., GRAHAM, L. T., APRISON, M. H. & WERMAN, R. (1967). Association of glycine with spinal interneurones. *Nature, Lond.*, **214**, 680–681.

FELDBERG, W. & SHERWOOD, S. L. (1954). Injections of drugs into the lateral ventricle of the cat. *J. Physiol., Lond.*, **123**, 148–167.

HENNEMAN, E., KAPLAN, A. & UNNA, K. R. (1949). A neuropharmacological study of the effect of myanesin (Tolserol) on motor system. *J. Pharmac. exp. Ther.*, **97**, 331–341.

HOPKIN, J. M. & NEAL, M. J. (1970). The release of ^{14}C-glycine from electrically stimulated rat spinal cord slices. *Br. J. Pharmac.*, **40**, 136*P*–138*P*.

KELLY, J. S. & KRNJEVIĆ, K. (1969). The action of glycine on cortical neurones. *Exp. Brain Res.*, **9**, 155–163.

KING, E. E. & UNNA, K. R. (1954). The action of mephenesin and other interneuron depressants on the brain stem. *J. Pharmac. exp. Ther.*, **111**, 293–301.

PHILLIS, J. W. (1970). *The Pharmacology of Synapses*, pp 223–230. London: Pergamon Press.

SHARMA, J. N., GUPTA, P. P. & DHAWAN, B. N. (1971). Some further evidence for a possible neurotransmitter role of glycine. *Ind. J. Pharmac.*, **3**, 21.

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