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NONSPECIFIC DEPRESSANT ACTION OF γ-AMINOBUTYRIC ACID ON SOMATIC REFLEXES

BY

K. P. BHARGAVA AND R. K. SRIVASTAVA*

From the Upgraded Department of Pharmacology and Therapeutics, K. G. Medical College, Lucknow, India

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The effects of γ-aminobutyric acid (GABA) on central integration of somatic reflexes in the cat have been studied by topical application, and by intrathecal or intracerebroventricular injection. Intrathecal injection of GABA inhibited the monosynaptic patellar reflex. The facilitation of the patellar reflex induced by strychnine, leptazol, tubocurarine and tetanus toxin was also inhibited. Polysynaptic facilitation of the patellar reflex induced in the spinal cat by electrical stimulation of the contralateral sciatic nerve was depressed by intrathecal GABA. Similarly, the supraspinal facilitation of the patellar reflex by electrical stimulation of the brain stem reticular formation was inhibited by application of GABA to the floor of the 4th ventricle. The polysynaptic inhibition of the patellar reflex at both levels was intensified by GABA. The flexor (tibialis anterior) reflex was depressed in the same manner as the extensor patellar reflex. The polysynaptic linguomandibular reflex was depressed by intracerebroventricular GABA. The depressant action of GABA at spinal and supraspinal levels of the neuraxis is discussed in relation to the role of GABA as an inhibitory transmitter in the central nervous system.

γ-Aminobutyric acid (GABA) is selectively present in the central nervous system. Topical application of GABA on the mammalian cerebral cortex inhibits electrically or chemically evoked activity (Hayashi & Nagai, 1956; Killam & Killam, 1958; Hayashi, 1960; Rech & Domino, 1960). Purpura, Girado, Smith & Gomez (1958) found that in the cat systemically administered GABA reduced the frequency of spontaneous paroxysmal discharge recorded from a site where the blood-brain barrier had been broken. Chemically-induced convulsions in mice were inhibited by GABA on topical or intracerebral application (Gulati & Stanton, 1960), by subcutaneous injection (McLennan, 1957), and by oral administration (Hawkins & Sarett, 1957), and in dogs by intracarotid injection (Hayashi, 1959). Inhibition has also been observed in the neuromuscular transmission of the crayfish claw preparation (Florey, 1956; McLennan, 1957), in the transcallosal pathways (Marrazzi, Hart & Rodriguez, 1958), in reticular pathways (Grundfest, 1960) and in spinal neurons (Curtis, 1961).

An inhibtory transmitter role for GABA has been suggested (Kuffler & Eyzaguirre, 1955; Elliot & Florey, 1956; Iwama & Jasper, 1957; McLennan, 1957; Vander Kloot, 1960), but evidence for such a role is not unequivocal. Recently Eccles (1962) has ruled out a specific inhibitory transmitter role of GABA since it failed to produce a hyperpolarization of the motoneuronal membrane.

* Present address: Defence Research Laboratories, Kanpur, India.

Topical application of GABA on the brain-stem of rabbits was found to facilitate the flexor reflex but the patellar extensor reflex remained unchanged; such an action was not observed on topical application of GABA on the spinal cord in spinal animals (Yamazaki, 1959).

The present study was undertaken to investigate the effect of GABA on the central integration of somatic reflexes both at the spinal and the supraspinal levels in cats.

METHODS

Cats of either sex weighing between 2.4 and 4.0 kg were used. All surgical procedures were done on cats anaesthetized with ether and subsequently the animals were maintained on light chloralose anaesthesia (50 mg/kg, intravenously). In spinal transected (C7) preparations, only 40 mg/kg of chloralose was injected. All the animals were maintained on artificial positive pressure ventilation and were vagotomized.

Drugs were dissolved in 0.9% saline. Intrathecal injection was given through a hypodermic needle introduced at the lumbosacral articulation. Intracerebroventricular injection was made according to the technique of Feldberg & Sherwood (1954). For topical application of GABA on the medulla, solutions of up to 0.5 g/ml. were prepared and a cotton pledget soaked in such a solution was kept on the calamus scriptorius for 5 to 10 min. Cotton pledgets soaked in 0.9% saline were used as controls. In some experiments the blood pressure was recorded from a carotid artery by means of a mercury manometer.

The somatic reflexes were elicited after 60 to 90 min from administration of the chloralose. The patellar monosynaptic reflex was elicited by tapping the patellar tendon by means of an electromagnetic hammer (every 10 sec) and recorded through a system of pulleys on a kymograph (Calma & Wright, 1947). Monosynaptic inhibition of the patellar reflex was elicited by stimulating the ipselateral sciatic nerve; the nerve of the opposite side was cut to avoid contralateral influences (Abdulian, Martin & Unna, 1960). Polysynaptic facilitation and inhibition of the patellar reflex were obtained by electrical stimulation of the contralateral sciatic nerve and the brain-stem reticular formation (Henneman, Kaplan & Unna, 1949). A concentric needle electrode was employed to stimulate the medullary reticular formation. The flexor reflex was recorded from the contractions of the tibialis anterior muscle produced by stimulation of the sciatic nerve distal to the origin of the nerve to the tibialis anterior muscle of the same side (Witkin, Spitaletta & Plummer, 1960). The polysynaptic linguomandibular reflex was obtained by stimulating the root of the tongue according to the method of King & Unna (1954). All stimuli were derived from a Grass Model S4 electronic stimulator delivering rectangular wave pulses.

RESULTS

Effect of GABA in spinal transected (C7) cats

The patellar reflex was elicited in sixteen spinal cats. Intrathecal injection of small doses of GABA (1 to 50 mg) did not affect the patellar reflex, while higher doses (60 to 100 mg) consistently depressed it. However, doses above 100 mg were required to depress the patellar reflex facilitated by prior intrathecal administration of tetanus toxin or tubocurarine and by intravenous injection of leptazol or strychnine. Previous intrathecal treatment with GABA (100 to 200 mg) also prevented the potentiation of the patellar reflex by these agents. In Fig. 1 are shown the effects of intrathecal GABA (100 mg) on the inhibition of patellar reflex due to ipselateral sciatic nerve stimulation (0.2 V, 120 shocks/sec for 10 sec). At 5 min after the administration of GABA, the patellar response was reduced and the inhibition of the reflex due to sciatic nerve stimulation was more pronounced. Recovery was apparent at 25 min.

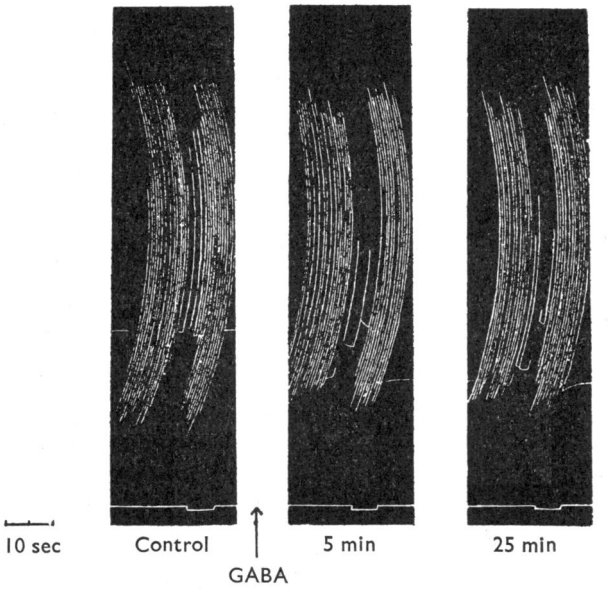

Fig. 1. Record of patellar tap response, elicited every 10 sec and the inhibitory effect of ipselateral sciatic nerve stimulation (0·2 V, 120 shocks/sec for 10 sec). The first panel shows the control response. Note, in the middle panel, the reduction in the patellar response and the more conspicuous inhibition to nerve stimulation after 5 min of intrathecal GABA (100 mg). Partial recovery occurred at 25 min after GABA. Time and calibration, 10 sec.

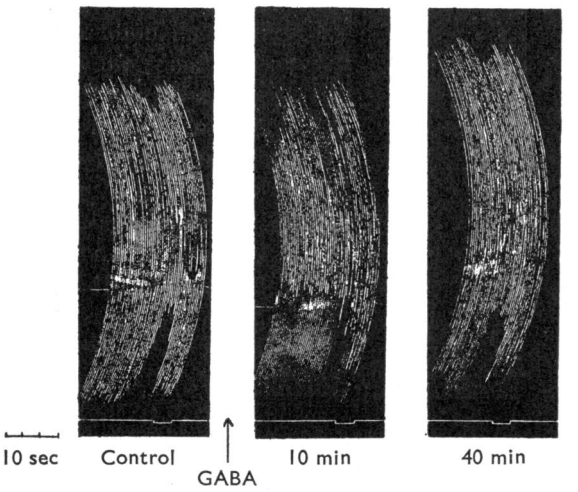

Fig. 2. Record of patellar tap response, every 10 sec and the (polysnaptic) inhibitory effect of contralateral sciatic nerve stimulation (9 V, 120 shocks/sec, for 10 sec). Note that intrathecal GABA (50 mg) selectively intensified the inhibition of the patellar response to nerve stimulation. Recovery was complete at 40 min.

The effects of GABA on the polysynaptic inhibition of the patellar reflex due to contralateral sciatic nerve stimulation (9 V, 120 shocks/sec for 10 sec) are shown in Fig. 2. Intrathecal GABA (50 mg) did not significantly reduce the patellar reflex response (at 10 min), while the inhibition during nerve stimulation was a little greater. Complete recovery of the response had occurred at 40 min.

In five spinal cats, polysynaptic facilitation of the patellar reflex was obtained by stimulation of the contralateral sciatic nerve (3 to 5 V, 120 shocks/sec for 10 sec). Each time intrathecal GABA (60 to 100 mg) reduced the patellar reflex response and antagonized the facilitation produced by nerve stimulation.

The effect of intrathecal GABA on the tibialis anterior muscle (flexor reflex) response to nerve stimulation was studied in three spinal cats. Fig. 3 shows the results of such a study. Intrathecal GABA (200 mg) gradually reduced the response

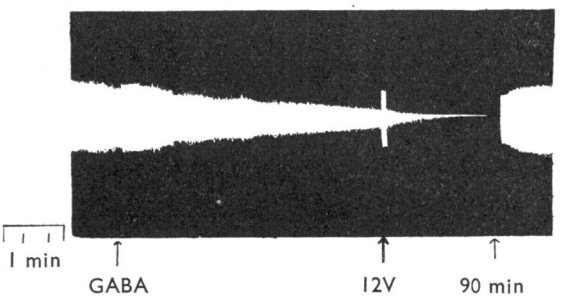

Fig. 3. Record showing tibialis anterior muscle response (flexor reflex) to ipselateral sciatic nerve stimulation distal to the origin of the nerve to the muscle (8 V, 1 shock/sec) in a spinal transected (C7) cat. Intrathecal administration of 200 mg of GABA at first arrow produced a gradual reduction of the response but nerve stimulation at higher voltage (120 V, at the second arrow) restored the original response. Recovery occurred after 90 min of the rest period (third arrow).

to nerve stimulation (8 V, 1 shock/sec), but the stimulation of the nerve with a higher voltage (12 V, 1 shock/sec) elicited a response almost equal to the control. The response was completely abolished within 40 min of GABA administration. Recovery occurred after a rest period of 90 min.

Supraspinal effect of GABA in intact cats

Effect of intracerebroventricular injection of GABA (10 to 20 mg) on the patellar reflex was studied in four cats. In Fig. 4 are shown the results of one such experiment. Injection of GABA (10 mg in 0.2 ml. of 0.9% saline) significantly depressed the patellar reflex in 10 min and complete recovery had occurred at 70 min.

Similarly, GABA (10 to 20 mg), injected intracerebroventricularly, depressed the linguomandibular reflex response to stimulation of the root of the tongue (2 V, 1 shock/sec) in six cats. Injection of 0.2 ml. of 0.9% saline did not have any effect on the reflex.

Polysynaptic facilitation of the patellar reflex was elicited in three cats by stimulation of the brain-stem reticular formation (3 to 4 V, 100 shocks/sec). Fig. 5 shows

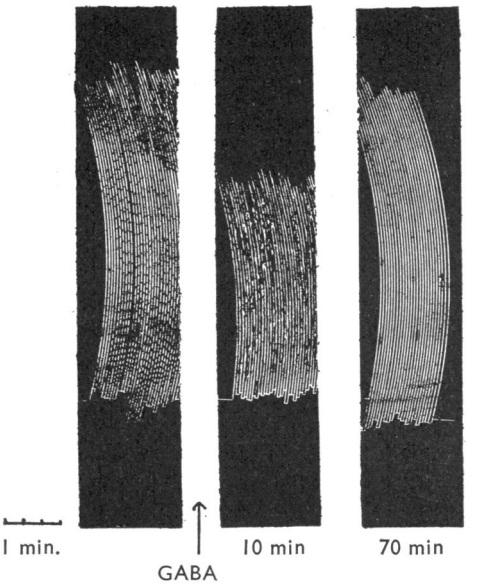

Fig. 4. Effect of intracerebroventricular GABA on the patellar tap response (every 10 sec). First panel shows the control response. Middle panel shows the clear depression of the response at 10 min after GABA (10 mg). Recovery occurred at 70 min.

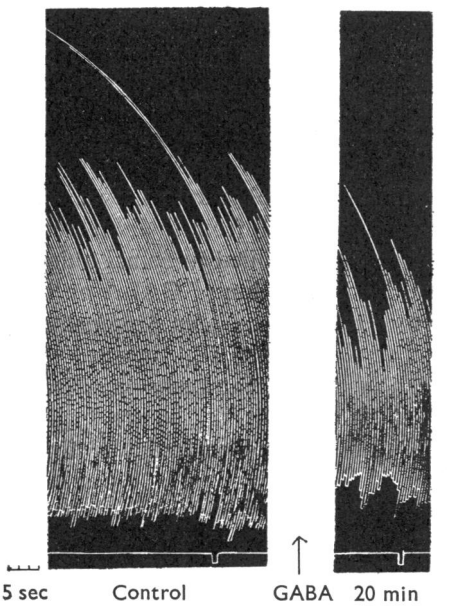

Fig. 5. Record of patellar tap response, every 10 sec, and the (polysnaptic) faciliation of the response to stereotaxic stimulation of the brain stem reticular formation (4 V, 100 shocks/sec for 3 sec). There was topical application of GABA (0·5 g/ml.) on the calamus scriptorius between panels. Note the depression of the patellar response and the reticular facilitation at 20 min.

the results of one such experiment. The electrode placement was done by means of a stereotaxic instrument (co-ordinates: anterior 12 to posterior 6 mm, lateral 4 to 5 mm and vertical −1 to 5 mm; Horsley-Clarke co-ordinates, see also Henneman *et al.*, 1949). Topical application of GABA (0.5 g/ml.) was made by placing a cotton pledget on the exposed calamus scriptorius. In the control panel, facilitation of the patellar reflex was elicited by stimulation of the reticular formation for 3 sec. The patellar reflex as well as its facilitation due to reticular stimulation was reduced after 20 min of topical application of GABA. Concentrations down to 5% produced similar effects, so that we do not consider the hypertonicity of the solution to be responsible.

Similarly, polysynaptic inhibition of the patellar reflex was elicited in four cats by stimulation of the brain-stem reticular formation (0.4 to 0.6 V, 100 shocks/sec). The electrode placement was done according to the following stereotaxic co-ordinates: posterior 8 to 10 mm, lateral 0 to 2 mm and vertical −5 to −8 mm. Local application of GABA (0.5 g/ml.) on the calamus scriptorius reduced the magnitude of the patellar reflex while the inhibition of the patellar reflex obtained by reticular stimulation was more pronounced at 20 min. Recovery of the responses occurred in 60 min.

DISCUSSION

The influence of the blood-brain barrier was excluded in the present study by localizing the GABA in the structures of the central nervous system by suitable routes of administration. The cat is much less sensitive to GABA than are the rabbit and the dog (Elliot & Hobbiger, 1959).

In order to be certain of the depressant action of GABA, comparatively high doses were employed in this study. McLennan (1957) observed that GABA (5 drops of 100 mg/ml.) did not inhibit the patellar reflex when the solution was applied to the exposed spinal cord of a decerebrate cat. From this observation the author concluded that GABA did not act at the spinal level. Similarly, the spinal site of action was denied by Yamazaki (1959) from the observation that the patellar monosynaptic extensor reflex was not inhibited in spinal animals. In the present study, however, a definite spinal locus of action of GABA on the monosynaptic and polysynaptic flexor and extensor reflexes has been demonstrated. This might be due to the employment of effective doses of GABA injected intrathecally. A depressant action of GABA was seen in the present study on reflexes integrated at the supraspinal level (linguomandibular reflex, inhibition and facilitation of the patellar reflex produced by stimulation of the brain-stem reticular formation, see Fig. 5), and a similar depressant action of GABA was observed on the monosynaptic and polysynaptic extensor and flexor reflexes integrated at the spinal level (Fig. 1, 2 and 3). Curtis, Phillis & Watkins (1959) also reported a depressant action of GABA on spinal neurones.

The observed effects could not be secondary to circulatory changes since adequate care was taken to exclude this factor and the blood pressure was high in most experiments. Since the GABA was localized in the structures of the central nervous system a peripheral action on somatic afferent receptors is quite unlikely. It cannot

be stated whether the inhibitory action of GABA was directly on the motor neurones or whether it was mediated through an action on central synapses such as the Renshaw system or the reticular system. A nonspecific depressant action of GABA on central synapses is more likely because of a uniform depressant action of the agent at the brain stem reticular level as well as at the spinal segmental level.

An antagonism between GABA and strychnine was also observed in the present study. This might apparently support the inhibitory transmitter role of GABA (Eccles, 1962). However, GABA showed an inhibitory action on the facilitations produced by diverse means. Chemically-induced facilitation of somatic reflexes integrated at both the spinal and the supraspinal levels with agents like leptazol, tubocurarine and tetanus toxin were inhibited by GABA. Electrically induced facilitation and inhibition of the patellar reflex through polysynaptic pathways (elicited by stimulation of the contralateral sciatic nerve and the brain stem reticular formation) were blocked by topical application of GABA. Furthermore, the monosynaptic inhibition of the patellar reflex due to stimulation of the ipsilateral sciatic nerve was also depressed (Fig. 1). A general depressant action of GABA at all levels of the neuraxis, observed in the present study, reaffirms a nonspecific inhibitor action of GABA.

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