ANTAGONISM OF THE SPINAL ACTION OF DIAZEPAM BY SEMICARBAZIDE

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In spinal unanaesthetized cats, pretreatment with semicarbazide (200 mg/kg) 2-4.5 h prior to the administration of diazepam (1-4 mg/kg) completely blocked the enhancement of the segmental dorsal root reflex by the latter compound. Pyridoxine hydrochloride (200 mg/kg), given 3.5 h after semicarbazide, restored the spinal effect of diazepam administered 1 h later. The possibility of a link between diazepam and spinal γ-aminobutyric acid is suggested and discussed.

The proposal of Eccles, Schmidt & Willis (1963) that γ-aminobutyric acid (GABA) may play an important role in the generation of spinal dorsal root potentials and reflexes has been corroborated by studies on the GABA antagonist, bicuculline, which reduces primary afferent depolarization of amphibian (Davidoff, 1972) and mammalian (Curtis, Duggan, Felix & Johnston, 1971; Levy & Anderson, 1972) spinal presynaptic terminals.

A recent study of possible interaction between GABA and pentobarbitone precluded the involvement of this inhibitory amino acid in the spinal depressant action of barbiturates, but pointed to a probable relationship in their effect on dorsal root potentials (Banna, 1973a). Diazepam (Valium, Hoffmann-LaRoche, Inc.) enhances the activity of the spinal presynaptic inhibitory pathway (Schmidt, Vogel & Zimmermann, 1967; Schlosser, 1971; Stratten & Barnes, 1971). Since it affects the dorsal root reflex (DRR) more selectively than other depressants, the presence of GABA may be an important factor in its spinal action. In this report, the effects of diazepam on spinal synaptic transmission in cats pretreated with semicarbazide are described.

Methods All experiments were performed on adult cats the spinal cord of which was transected at the atlanto-occipital junction under ether. Anaesthesia was then discontinued and artificial respiration instituted. Both carotid arteries were ligated, and manual pressure was applied to the vertebrals to make the brain ischaemic. The lumbosacral spinal cord was exposed by laminectomy, the dura sectioned, and a pool of paraffin oil (maintained at 37° C by thermostatic control) was prepared to cover nerve tissue. A heating pad placed under the cat kept body temperature near 37° C. All roots from L_5 to S_2 were sectioned extradurally. Bipolar platinum hook electrodes were placed on dorsal and ventral L_7 roots and on a dorsal L_6 strand. Supramaximal square wave stimuli were applied to dorsal L_7 root at a rate of 0.25 Hz, and the evoked ventral root and antidromic dorsal root discharges were monitored from ventral L_7 and dorsal L_6 roots, respectively. Blood pressure was continuously recorded from a carotid artery, and drugs were administered through a cannulated antebrachial vein. All the animals were immobilized by gallamine triethiodide, and end tidal levels of CO_2 were maintained between 3 and 4%. Diazepam was administered in a special solvent medium supplied by the manufacturer. The solvent exerted no effects when administered separately in quantities similar to those used in the diazepam experiments. The area of the DRR recorded from the dorsal root was integrated by planimetry. A more detailed account of the techniques used is given elsewhere (Banna & Jabbur, 1972).

Results In control experiments, diazepam (1 mg/kg) selectively and reliably enhanced the DRR in those preparations where such a record could be obtained. The net increase averaged 121 ± 8%. More importantly, it induced the appearance of a DRR in two preparations where such a reflex could not initially be recorded at normal body and oil pool temperatures. In other experiments, diazepam was administered at various intervals following semicarbazide (200 mg/kg) pretreatment (Table 1). The ability of diazepam to increase the size of the evoked DRR persisted when it was given within 1 h of, semicarbazide, but this effect, even following doses as high as 4 mg/kg, was greatly reduced at 1.5 h and finally blocked completely 2-4.5 h later. Semicarbazide itself reduced and then abolished the DRR within 2 h, but diazepam did not re-establish this synaptic process as it did in control cats where no DRR could be initially recorded. Diazepam also failed to

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increase the size of the dorsal root potential 2 h after semicarbazide. In three experiments, pyridoxine hydrochloride (200 mg/kg) was administered 3.5 h after semicarbazide, and within 1 h, diazepam (1 mg/kg) again increased the size of the DRR by 173 ± 15%, showing complete recovery of action. Pyridoxine also antagonized the depression of the DRR induced by semicarbazide, bringing the size of this reflex back to near control values within 1 h (Table 1). Finally, when semicarbazide was administered 5-10 min after diazepam, at a time when the full effects of the latter compound had developed, it failed to antagonize these effects.

Discussion These results invite a correlation between the spinal action of diazepam and lumbosacral GABA levels. Although semicarbazide belongs to a group of compounds known for their carbonyl trapping properties, it does not appear that direct chemical interaction occurs between it and diazepam, since no antagonism could be demonstrated at early time intervals. This failure of antagonism within the first hour also rules out a specific receptor type of blockade. Bell & Anderson (1972) showed that semicarbazide gradually depletes lumbosacral GABA in spinal cats, and that within 2 h, less than 50% of control GABA levels remains. Although a nonselective effect of semicarbazide on neurohumoural agents cannot be ruled out, its ability to deplete GABA in the central nervous system appears to be reasonably specific, since it mainly inhibits the activity the CNS appears to be reasonably specific, since it mainly inhibits the activity of glutamate decarboxylase after *in vivo* administration (Killam & Bain, 1957). On the other hand, pyridoxine has been shown to antagonize effects of semicarbazide on presynaptic inhibition within 1 h, after a corresponding repletion of GABA in nerve tissue (Banna, 1973b). It thus appears that the presence of normal levels of GABA is essential for the manifestation of the spinal action of diazepam, which in turn may partly underly its muscle relaxant (Schmidt *et al.*, 1967; Hudson & Wolpert, 1970; Schlosser, 1971) and anticonvulsant effects. It also appears that the ability of diazepam to enhance the DRR is not the result of a direct action on afferent presynaptic terminals, otherwise its effects would have persisted after GABA depletion.

Table 1 Effect of diazepam (1 mg/kg) on the dorsal root reflex (DRR) in spinal cats

Pretreatment	*Effect (%)*
None	121 ± 8 (5)*
Semicarbazide (5-10 min)	130 ± 23 (4)
Semicarbazide (0.5 h)	163 ± 21 (3)
Semicarbazide (1.5 h)	**
Semicarbazide (2 h, 3.5 h, & 4.5 h)	0 (6)
Semicarbazide (3.5 h) + pyridoxine (1 h)	173 ± 15 (3)

* Mean increase in DRR area, followed by standard error and number of experiments in parentheses.
** The actual increase in DRR size induced by diazepam at this interval was very small. It is not reported as a percentage here since it would appear disproportionately large due to the pronounced decrease in DRR size induced by semicarbazide.

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