Locus of Central Depressant Action of Some Benzodiazepine Analogues[1] (35613)

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Chlordiazepoxide (Librium) was the first compound of a new class of benzodiazepines that was found to have muscle relaxant as well as antianxiety activity (8). Another analogue, diazepam (Valium), was reported to be about 20 times more potent than chlordiazepoxide in blocking rigidity in decerebrate cats (9, 10). It was shown that these agents depress the spinal polysynaptic extensor reflexes but not the monosynaptic knee jerk reflex in midcollicular decerebrate cats (6). However, it was also noted that they were less effective in depressing the polysynaptic reflexes in spinal preparations. Przybyla and Wang (7) reported that diazepam depressed facilitation and inhibition of the knee jerk induced by stimulation of the brainstem reticular formation. These results indicate that the primary action of diazepam is at the supraspinal level, most likely on the brainstem reticular formation. Four other benzodiazepine analogues, Ro 5-4023, Ro 5-3059 (nitrazepam), Ro 5-3350, and Ro 5-6901 (Dalmane), have also been reported to abolish decerebrate rigidity in cats (14). However, their selective sites of action have not yet been demonstrated. This study utilized the techniques of transection and stimulation to define the sites of primary depression produced by these four agents.

Methods. Cats of both sexes weighing 2 to 4.5 kg were used. After tracheostomy, midcollicular decerebration was performed under diethyl ether anesthesia by a modified Sherrington method (13). Arterial blood pressure was measured in the left common carotid artery with a Statham P23AC transducer and recorded on a Grass Model 7 polygraph. The radial vein was cannulated for drug injection. Body temperature was monitored with a rectal thermometer and maintained at 37 to 39° with an electric heating pad or an infrared heating lamp.

A. Transection experiments. To elicit ipsilateral and contralateral polysynaptic extensor reflexes, the central end of the cut left sciatic nerve was stimulated electrically with a bipolar electrode. Contractions of both right and left quadriceps femoris (reflex responses) were monitored with Grass FT-03 force-displacement transducers attached to the patellar tendons. The femurs were rigidly fixed with transcondylar pins. Stimuli are isolated rectangular pulses 2–5 V in intensity and 1 msec in duration, delivered at a frequency of 0.5/sec. The spinal cord was exposed at the level of the first cervical segment for subsequent transection. Lidocaine was applied to the surface of the exposed spinal cord before transection, in order to avoid muscular movement and hypertensive reaction at the time of transection. Artificial ventilation was provided.

B. Stimulation experiments. In midcollicular decerebrate cats, the monosynaptic patellar reflex (knee jerk) was elicited by tapping the patellar tendon with a Palmer hammer at 1/sec. It was monitored with a Grass FT-03 force displacement transducer fastened to the heel. The femur was fixed as described above. Electrical stimulation of the lateral mesencephalic tegmentum or the ventromedial medullary reticular formation, respectively, facilitated or inhibited the knee jerk. These areas are known to be located within the reticular facilitatory and inhibitory systems (RFS and RIS) of Magoun and

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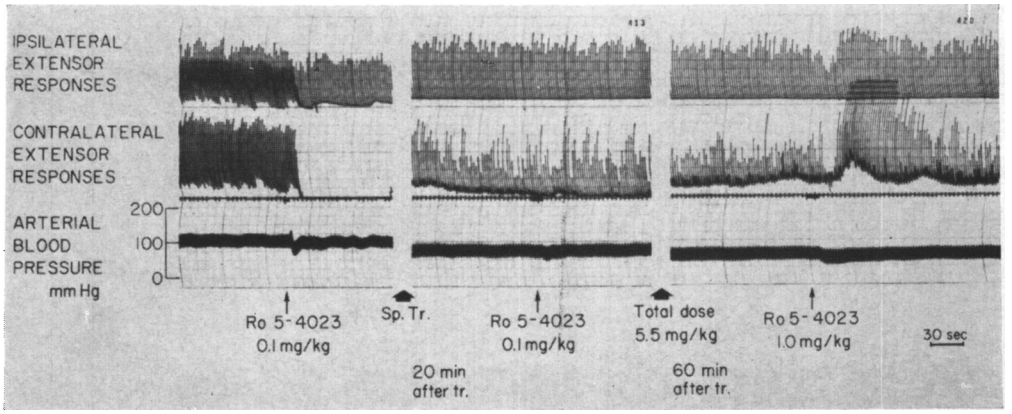

FIG. 1. Midcollicular decerebrate cat; effects of Ro5-4023 on ipsilateral and contralateral extensor reflex responses before and after spinal transection: wt, 3.3 kg; stimulus parameters: 2V, 1 msec in duration and 0.5/sec one hundred min after decerebration, 0.1 mg/kg of Ro 5-4023 was injected iv. Ipsilateral extensor responses were minimally affected, while contralateral responses were abolished. Spinal cord was transected at C_1 while contralateral responses remained depressed. Five min later, the ipsilateral responses became exaggerated and the gain was reduced to half. The contralateral responses reappeared but were smaller than control and the gain was doubled. Note that after total cumulative dose of 6.6 mg/kg of Ro 5-4023 had been given, the reflex responses were not depressed.

Rhines (5). Stimulating currents of 0.03–0.15 mA and 1 msec duration, from a Grass constant current unit were applied for 10-sec periods. The effect of stimulation on the knee jerk was recorded before and after intravenous administration of drugs.

Anatomic location of the stimulating electrode was confirmed histologically. Lesions were made in the brainstem substance by passing 2 mA dc for 30 sec through the electrode. The head was then perfused with 10% formalin. The brain was frozen, sectioned, and stained by the Weil method.

Results. A. Transection experiments. 1. Ro 5-4023. In 5 midcollicular preparations, intravenous administration of Ro 5-4023 in doses of 0.05 to 0.15 mg/kg always abolished the contralateral extensor reflex (Fig. 1). This drug usually reduced the resting tone of the contralateral quadriceps femoris in these preparations. The ipsilateral extensor reflexes were relatively resistant to Ro 5-4023; the depression varied from 10 to 100% within the same dose range. In two other midcollicular preparations the contralateral extensor reflexes appeared to be very resistant to the drug. In one of them, a dose of 1 mg/kg produced only transient depression of reflexes which recovered to control level within 10 min.

After subsequent transection at the spinal C_1 level, the ipsilateral extensor reflex recovered and often became greater than the original control. The contralateral extensor reflex also reappeared but was usually smaller than the control. In the same spinal preparations, Ro 5-4023 (0.05–0.15 mg/kg) did not depress either the ipsilateral or the contralateral extensor reflexes. Higher doses of this drug (0.5 to 5 mg/kg) produced transient facilitation of both reflexes.

2. Ro 5-3059. In 3 midcollicular preparations, 0.1 mg/kg of Ro 5-3059 (nitrazepam) abolished the contralateral extensor reflex and reduced the ipsilateral extensor reflex to 0 to 80% of control. In another midcollicular animal, the extensor reflexes were resistant to the drug. A cumulative dose of 0.6 mg/kg did not abolish the contralateral extensor reflex. Again after spinal transection, the reflexes recovered. Both extensor reflexes were relatively resistant in spinal preparations. In one of these animals, higher doses of this drug (2–5 mg/kg) produced transient facilitation of extensor reflexes, as in the case of Ro 5-4023.

3. Ro 5-3350. In 4 midcollicular preparations, 0.2 to 0.5 mg/kg of Ro 5-3350 abol-

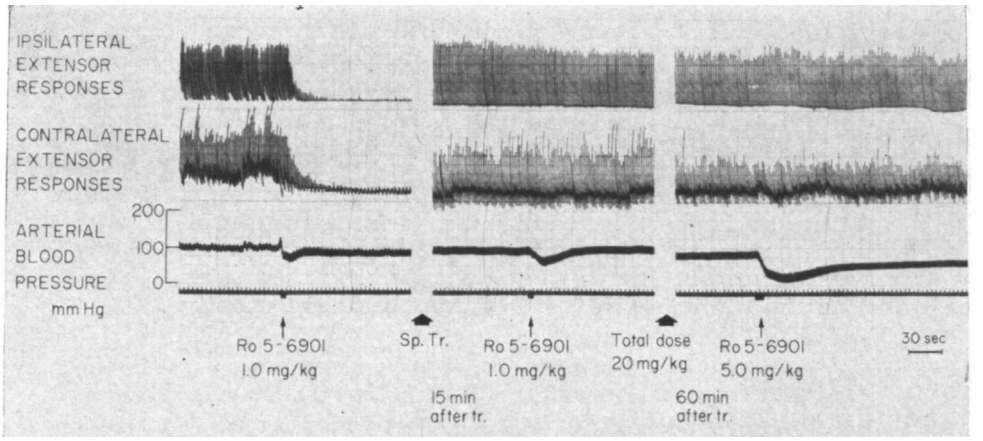

FIG. 2. Midcollicular decerebrate cat; effects of Ro 5-6901 on ipsilateral and contralateral extensor reflex responses before and after spinal transection: wt, 3.2 kg. Stimulus parameters: 3.8 V, 1 msec in duration and 0.5/sec. one hundred min after decerebration, 1 mg/kg of Ro 5-6901 was injected iv. Both ipsilateral and contralateral reflex responses were abolished. Ten min after spinal cord was transected at C_1 level, both reflex responses recovered. The gain for ipsilateral responses was reduced to 1/5 of control. The gain for contralateral responses was doubled. Administration of total cumulative dose of the drug, 26 mg/kg, only slightly depressed the reflex responses.

ished the contralateral extensor reflex. The ipsilateral reflex was reduced to 0 to 50% of control. In another animal, the extensor reflexes proved to be very resistant to this analogue. Cumulative dosage of 3 mg/kg did not abolish the extensor reflexes. After spinal transection, the reflexes were more resistant to this analogue.

4. Ro 5-6901. Similar results were observed with Ro 5-6901 (Dalmane) in 4 midcollicular preparations. This analogue, however, was the least potent muscle relaxant among the four benzodiazepine compounds studied here. With 1 to 1.5 mg/kg of Ro 5-6901, given iv, the contralateral reflex was abolished and the ipsilateral reflex was reduced to 25% of control (Fig. 2). After spinal transection, a cumulative dose of 26 mg/kg did not abolish the extensor reflexes.

B. Stimulation experiments. The effects of these 4 benzodiazepine compounds on the brainstem reticular system were studied in 10 midcollicular cats. The mesencephalic reticular facilitatory system and medullary reticular inhibitory system were stimulated and the resulting facilitation and inhibition of the knee jerk was recorded before and after iv administration of the drugs. Doses of these benzodiazepines which abolished the spinal contralateral extensor reflex always depressed both facilitation and inhibition. The doses used for each drug were: 0.1 mg/kg for Ro 5-4023, 0.2 mg/kg for Ro 5-3350, 0.1 mg/kg for Ro 5-3059, and 1 mg/kg for Ro 5-6901. Increases above these doses caused further depression of the facilitation or inhibition. Figures 3 and 4 provide examples of these phenomena. The knee jerk itself was not influenced by the dose of drug which caused profound depression of the facilitatory and inhibitory effects.

In some preparations, facilitation of the knee jerk caused by stimulation of mesencephalic reticular facilitatory system was associated with an increase of basal tone. The drugs inhibited the elevating basal tone as well as facilitation of the knee jerk.

Intravenous administration of equivalent volumes of propylene glycol, vehicle for these diazepam analogues, did not yield any observable change in either transection or stimulation experiments.

Discussion. The mechanism of action of mephenesin, one of the centrally acting skeletal muscle relaxants, was extensively studied more than 2 decades ago. It was believed that this agent selectively depressed spinal interneurons since it reduced certain

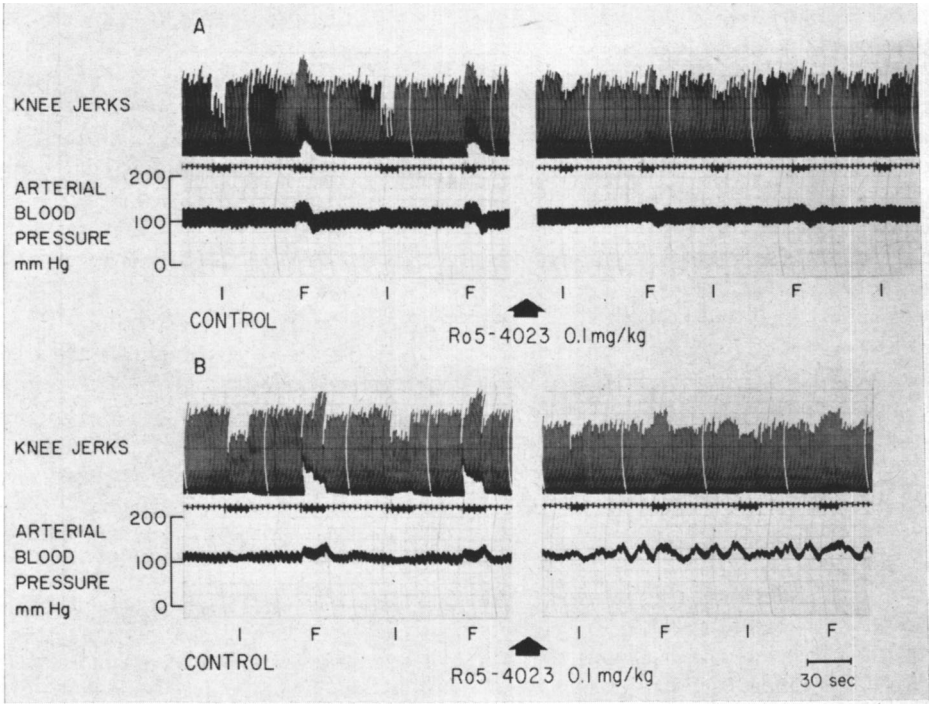

FIG. 3. Midcollicular decerebrate cats; effects of Ro 5-4023 on the mesencephalic facilitatory (F) and medullary inhibitory (I) influences of the knee jerks in the midcollicular cats: Two cats are shown: (upper traces) knee jerk responses showing F and I. (bottom traces) carotid arterial pressure: (A) cat, 3 kg; constant current, 0.07 mA was applied for both F and I. The duration of stimulation was 10 sec. (B) Cat, 2.5 kg; constant current, 0.15 and 0.07 mA, was applied for F and I, respectively. In both preparations, both F and I were depressed by Ro 5-4023.

polysynaptic but not monosynaptic spinal reflexes (1, 2). The effects of mephenesin are not limited to the spinal level. Several investigators (2–4) reported that mephenesin decreased or abolished facilitation and inhibition of the anterior horn cells produced by CNS stimulation at different levels. Henneman *et al.* (2) concluded that drug action at

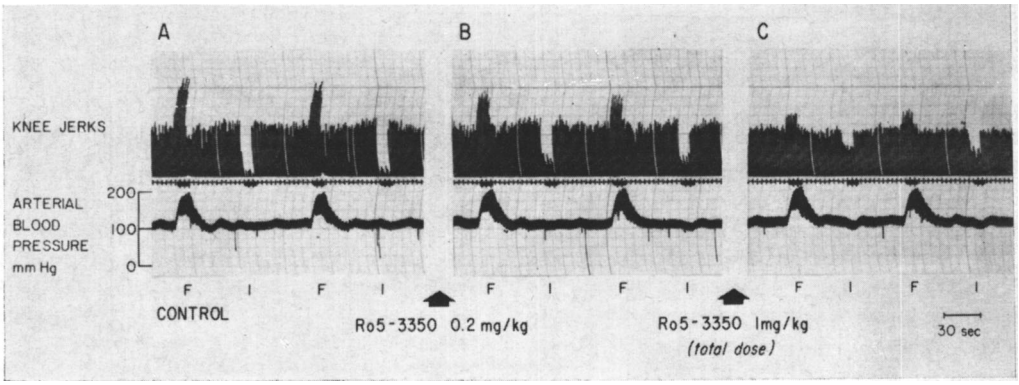

FIG. 4. Midcollicular decerebrate cat; effects of Ro 5-3350 on the mesencephalic facilitatory (F) and medullary inhibitory (I) influences of the knee jerks in midcollicular cats: wt, 3.3 kg; constant current, 0.03 mA, was applied for both F and I. The duration of stimulation was 10 sec. Both F and I were depressed by Ro 5-3350.

the spinal level alone was sufficient to explain all effects on spinal polysynaptic reflexes though they might likewise be explained by an action at the other levels where internuncial cells exist. King and Unna (4) showed that the polysynaptic lingomandibular reflex, which involves only brainstem interneurons, was sensitive to mephenesin. However, the selective site of action of mephenesin and other muscle relaxants was not clearly demonstrated until Ngai *et al.* (6) showed that a much larger dose was required to block the polysynaptic spinal reflexes after spinal cord transection. With diazepam, it was found that a dose 100 times greater than that used in midcollicular preparations was required to depress polysynaptic reflexes in spinal preparations.

The present examination demonstrated that in midcollicular preparations the polysynaptic reflex responses were also susceptible to depression by four new benzodiazepine analogues. The reflexes, however, were much more resistant after spinal cord transection. The results were similar to those obtained with diazepam. The only differences were: the dosages required and also that there were a number of midcollicular preparations which were found to be quite resistant to the analogues. After spinal transection, the reflexes exhibited resistance to high doses of benzodiazepine analogues. Indeed, in the case of Ro 5-4023, high doses increased the muscle tone and the extensor reflexes. The increased muscle tone and extensor reflexes can be reduced by another type of muscle relaxant, tybamate (11, 12).

The present investigation indicates that the predominant site of action of these four agents is at the supraspinal level. In some midcollicular preparations, unlike diazepam, the new benzodiazepine analogues were relatively ineffective to depress the contralateral polysynaptic reflex. Although the reason for the resistance of polysynaptic reflexes to these benzodiazepine compounds in a few animals is not clear, the results obtained in the large majority of animals support the view that the site of depressant action of this group of drugs is supraspinal.

It is interesting to note that in midcollicular cats the ipsilateral extensor reflex was less sensitive to drugs. In addition, after spinal transection, the ipsilateral reflex was usually enhanced. It therefore appears possible that the ipsilateral reflex has a substantial monosynaptic component. Supraspinal structures, most likely those associated with the reticular system, may normally exert an inhibitory influence on the ipsilateral extensor reflex, like that on the knee jerk.

The stimulation experiments showed that these 4 agents, in doses that depress the polysynaptic reflexes, depressed both the mesencephalic reticular facilitatory and the medullary reticular inhibitory effect on the knee jerk. These observations were similar to those observed with diazepam (7), and further suggest the brainstem site of action of these agents.

Summary. In midcollicular decerebrate cats, four benzodiazepine analogues, Ro 5-4023, Ro 5-3059 (nitrazepam), Ro 5-3350, Ro 5-6901 (Dalmane) were found to be very effective in blocking spinal polysynaptic reflexes. Subsequent to spinal cord transection during the period of drug-induced depression, these reflexes returned and became highly resistant to depression by these drugs. With stimulation experiments, these four agents depressed both the reticular facilitatory and inhibitory effects on the knee jerk. It was concluded that these drugs act mainly on supraspinal structures, most likely in the brainstem reticular system.

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