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Metabolism and anticonvulsant activity of diazepam in guinea pigs

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PREVIOUS studies have shown that diazepam, incubated *in vitro* with liver microsomes of different animal species[1,2] is hydroxylated and *N*-demethylated in rats and mice while it is only *N*-demethylated in guinea pigs.

In an effort to compare the metabolism of diazepam with the anticonvulsant activity of this benzodiazepine a study was made for investigating these aspects in guinea pigs according to a pattern previously employed in rats and in mice.[3]

Male Albino guinea pigs (body wt. 300–350 g) were used in all experiments.

Diazepam was administered by intravenous injection at a dose of 5 mg/kg dissolved in a solvent containing propylglycol–glycofurol–benzyl alcohol–water (30:30:2:48). Metrazol was injected i.p. at different doses and at different times after administration of diazepam, as specified below.

Extraction of diazepam and metabolites and their gas chromatographic determination. The preparation of blood, brain and adipose tissue extracts were made according to the method previously reported.[4] Gas chromatographic analyses were carried out using a gas chromatograph Model G_1 (Carlo Erba, Milan) equipped with a Ni 63 electron capture detector (Voltage 42 V).

The stationary phase was OV_1 3% on Gas Chrom Q (60–80 mesh) packed into a 4 m glass column (int. dia. 2 mm; ext. dia. 4 mm).

The flow rate of the carrier gas, nitrogen, was 33 ml/min, the column temperature was 245° and the injection temperature was 290°.

At various times after i.v. administration of diazepam, metrazol was injected intraperitoneally beginning with a dose of 100 mg/kg and increasing it by a factor of 1,2. Groups of six guinea pigs were observed for a period of 30 min after metrazol injection and their responses recorded. The parameter used for measuring antimetrazol activity was the relative number of animals undergoing clonic and tonic seizures.

The protection against the convulsant action of graded amounts of metrazol by pretreatment with diazepam injected i.v. at a fixed single dose of 5 mg/kg is shown in Fig. 1. Metrazol was given i.p. at different times after diazepam administration. Different doses were used beginning with 100 mg/kg (a 100 per cent lethal dose for guinea pigs) and increasing the dose by a factor of 1,2.

The anticonvulsant activity exerted by diazepam against metrazol declines with the time after diazepam administration, so that no protection against the convulsive response was observed when metrazol was given 10 hr after the pretreatment with diazepam.

In Table 1 is reported the metabolic pattern of diazepam in blood, brain and adipose tissue of guinea pigs. The highest blood and brain concentrations of intact diazepam were observed at 5 min after diazepam administration, but in the adipose tissue the concentration of intact diazepam reached a peak level only 30 min after diazepam administration.

Thereafter, in all tissues there was a gradual decline. The blood levels of diazepam were undetectable at 10 hr while in brain and in adipose tissue there were still measurable levels 20 hr after the intravenous administration of diazepam.

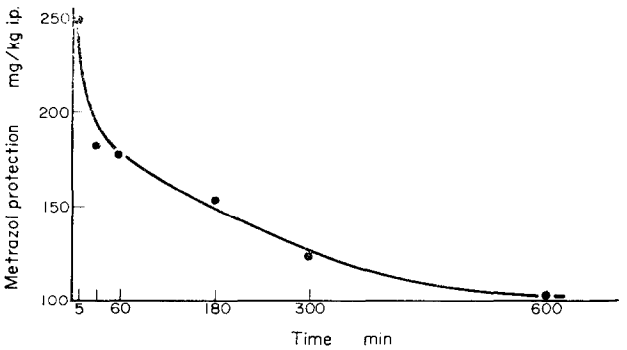

FIG. 1. Anticonvulsant effect of diazepam in guinea pigs. Diazepam (5 mg/kg i.v.) was given at the times shown on the abscissae prior to metrazol (doses reported on the ordinates). The points represent the dose of metrazol which was protected by at least 50 per cent. The parameter used for measuring antimetrazol activity was the percentage of animals undergoing clonic and tonic seizures. Metrazol was administered i.p. at graded doses beginning with 100 mg/kg and increasing the dose by a factor of 1,2. The dose of 100 mg/kg i.p. was lethal for 100 per cent of guinea pigs. One group of at least six animals was used for each dose of metrazol and each time.

TABLE 1. CONCENTRATIONS OF DIAZEPAM AND ITS METABOLITES IN PLASMA, BRAIN AND ADIPOSE TISSUE OF GUINEA PIGS AT VARIOUS TIMES AFTER AN INTRAVENOUS ADMINISTRATION OF DIAZEPAM (5 mg/kg)

Time after diazepam	Blood (μg/ml ± S.E.)			Brain (μg/g ± S.E.)			Adipose tissue (μg/g ± S.E.)		
	D	DD	OX	D	DD	OX	D	DD	OX
5 min	0·64 ±0·04	0·20 ±0·03	< 0·01	6·28 ±0·30	0·08 ±0·02	< 0·01	3·26 ±0·08	< 0·01	< 0·01
30 min	0·28 ±0·05	0·26 ±0·06	< 0·01	1·13 ±0·06	1·65 ±0·18	< 0·01	6·54 ±0·58	0·35 ±0·01	< 0·01
1 hr	0·16 ±0·04	0·37 ±0·07	< 0·01	0·45 ±0·02	1·46 ±0·13	< 0·01	3·66 ±0·31	1·78 ±0·17	< 0·01
3 hr	0·04 ±0·01	0·20 ±0·04	< 0·01	0·13 ±0·02	0·96 ±0·03	< 0·01	2·95 ±0·32	2·19 ±0·23	< 0·01
5 hr	0·02 ±0·004	0·08 ±0·006	< 0·01	0·08 ±0·01	0·60 ±0·05	0·03 ±0·004	1·45 ±0·09	1·73 ±0·06	< 0·01
10 hr	< 0·01	0·03 ±0·002	< 0·01	0·01 ±0·0001	0·18 ±0·002	0·06 ±0·006	0·68 ±0·02	0·89 ±0·09	0·07 ±0·01
20 hr	< 0·01	0·02 ±0·004	< 0·01	0·01 ±0·002	0·06 ±0·004	0·03 ±0·002	0·10 ±0·017	0·12 ±0·02	< 0·01
40 hr	< 0·01	< 0·01	< 0·01	< 0·01	< 0·01	< 0·01	< 0·01	< 0·01	< 0·01

D = Diazepam.
DD = *N*-demethyldiazepam.
OX = Oxazepam.

The major metabolite which accumulates in blood, brain and adipose tissue of guinea pigs after dministration of diazepam is the *N*-demethyldiazepam. In fact at 3 and 5 hr after diazepam administration, the concentration of the *N*-demethylmetabolite in the brain exceeds by several times the concentration of the parent drug.

Oxazepam does not accumulate in guinea pigs after administration of diazepam. Only very low levels are present at late times in the brain. The fact that oxazepam could not be detected in the tissues does not mean that it is not formed. In fact a rapid conjugation with glucuronic acid would permit a continuous elimination of oxazepam. However, it is interesting to underline that liver

microsomal enzymes of guinea pigs do not hydroxylate diazepam.[2] The anticonvulsant activity of diazepam in guinea pigs is therefore mostly correlated in the brain with the presence of diazepam and *N*-demethyldiazepam. In comparison to other animal species guinea pigs are different from either rats or mice in metabolizing diazepam *in vivo*. In fact guinea pigs are similar to mice in respect to accumulation of *N*-demethyldiazepam, but similar to rats for the lack of accumulation of oxazepam.

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Acetylcholine—A possible mechanism for the depolarization response in giant axons of the lobster circumesophageal connective*

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IT HAS been reported that the giant axons of the lobster circumesophageal connective respond to the external application of acetylcholine (ACh) with a membrane depolarization.[1,2] This phenomenon has been of particular interest in view of a proposed role for acetylcholine in axonal conduction.[3] The studies cited have indicated at least two categories of response to ACh in this population of axons: (1) depolarization resulting in an immediate decrease in excitability and spike decrement; and (2) depolarization which initiates spontaneous discharge. It has also been shown that cholinesterase inhibitors, depending on their concentration, fully or partially block these actions of ACh. In the present study we have further examined the relationship between cholinesterase activity and the ACh depolarization in an attempt to clarify a possible interaction and determine whether it reflects a functional role of acetylcholine in the conducted response.

The ligated desheathed connective was mounted in a perfusion chamber[4,5] that permits continuous flow of the bathing medium (2–4 ml/min) with a simple "nontraumatic" switching of solutions. The resting potential and action potentials were monitored via an intracellular glass microelectrode (3–10 megohms) and a conventional high input impedance preamplifier (Bioelectric NF-1). The output was displayed on an oscilloscope and the resting potential was recorded continuously on a strip chart recorder (Varian G-1000) connected to its cathode follower output. The nerve bundle was stimulated electrically with a bipolar electrode driven by a conventional stimulator and isolation unit (Grass). Artificial sea water[1] was the standard perfusion medium. Test substances were dissolved in it and the pH was adjusted to 7·7.

In the population of 7–10 larger giant axons of the circumesophageal connective, we have observed three basic responses to the application of acetylcholine (1×10^{-2} M). Some axons are insensitive even to this high concentration of ACh or respond with a slow depolarization only after sustained exposure (30–60 min). Others begin to depolarize at a moderate rate after 5–10 min of exposure with

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