Conflict behaviour in rats for the evaluation of a homogeneous series of 3-hydroxybenzodiazepines: structure-activity relationships

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The conflict behaviour in rats according to Geller (Geller & Seifter, 1960) was employed to investigate the structure-activity relationship of a series of nine benzodiazepines substituted in the N_1 position (H, CH_3 or CH_2CH_2OH) and in the ortho position of the phenyl ring (H, F or Cl).

Rats previously trained to a conflict behaviour schedule in conventional Skinner boxes were used. For each compound four doses were tested using 5-6 animals per dose level. The effects upon both the punished and non-punished response rates were taken into account.

The actions of the same series of drugs upon exploratory activity, cortical EEG, metrazol-induced seizures and inclined screen test were also investigated to control the concordance of the conflict behaviour with other tests for central activity.

It was found that: (1) Substitution into N_1 position with a methyl group increases the anxiolytic potency of the compound while a longer chain (CH_2CH_2OH) reduces it. (2) Halogen subsitution in the ortho position of the phenyl ring very strongly enhances the anxiety-reducing action whatever the N_1 substitution may be. (3) Fluorine seems equivalent to chlorine in determining the anti-anxiety potency of the compounds. (4) The results of the conflict behaviour test correlate well with those obtained in the other tests.

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Sleep and hypothermic effects of clonidine in fowls

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Clonidine (2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride) induces sleep when given intravenously to cats and young chickens (Zaimis, 1970; Holman, Shillito & Vogt, 1971) and when given intraventricularly to rats (Holman *et al.*, 1971). In sheep and goats intraventricular clonidine lowered or elevated body temperature, depending whether ambient temperature was below or above thermoneutrality (Maskrey, Vogt & Bligh, 1970).

The following experiments were performed in adult fowls at thermoneutrality. Given into the *IIIrd cerebral ventricle*, clonidine (0.02-0.4 μmol) induced behavioural and electrocortical sleep (15-30 min) followed by drowsiness, chickens squatting or standing; body temperature declined up to 2.0° C with recovery in 2-4 hours. These phenomena resembled those produced by intraventricular noradrenaline, 0.5 μmol; much more marked than with noradrenaline, respiratory rate increased to 60-150 min for 15-40 min and there was wing abduction. Changes in comb temperature, which increased as much as 10.0° C during the decline in body temperature, were reciprocally related to changes in body temperature. Arousal elicited by dexamphetamine (20 μmol/kg i.p.) was antagonized and replaced by sleep after intraventricular clonidine (0.1-0.4 μmol). *Infused into the hypothalamus*, clonidine (0.02-0.16 μmol) induced behavioural and electrocortical sleep lasting 60 min; body temperature declined up to 1.8° C with recovery delayed to 7 hours. With larger doses (0.1 μmol or greater) respiratory rate was elevated and associated with wing abduction. These effects of intraventricular clonidine (0.05 μmol) were prevented (intraventricular doses) by phenoxybenzamine (0.25 μmol) and substantially reduced by phentolamine (0.05 and 0.75 μmol), but were unaltered by propranolol (0.25 μmol), methysergide (0.1 μmol), atropine (0.4 μmol), or by pretreatment with p-chlorophenylalanine methyl ester. These doses of antagonists lacked intrinsic effects except for atropine which elevated body temperature 2.0° C-2.5° C; intraventricular phentolamine 0.135 μmol and 0.27 μmol also elevated body temperature, evoked shivering and wing abduction, and increased respiratory rate. Hyperthermic effects of intraventricular phenoxybenazine, and to a lesser extent phentolamine, have been observed in cats (Feldberg & Saxena, 1971).

In conclusion, sleep, previously noted by other workers, following intravenous clonidine in chicks, can be reproduced in adult fowls by its infusion into the IIIrd ventricle or hypothalamus; this and the fall of body temperature were due to an action of clonidine on brain α-adrenoreceptors. Although the effects resembled those of noradrenaline given intraventricularly or infused into the hypothalamus (Grunden & Marley, 1970; Marley & Stephenson, 1970; Marley & Nisticò, 1972), there were differences inasmuch as intraventricular clonidine much more obviously activated heat-loss mechanisms (panting, wing abduction and long-lasting peripheral vasodilatation).

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Synaptosomal exchange of γ-amino-butyric acid (GABA) can simulate high affinity uptake

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It is generally believed that inactivation of neurotransmitter amino acids occurs through high affinity uptake systems present in presynaptic nerve terminals (Iversen & Johnston, 1971; Snyder, Young, Bennett & Mulder, 1973). These uptake systems were demonstrated by measuring the radioactivity taken up by various nervous tissue preparations (including synaptosomes) from incubation media containing micromolar concentrations of radio-labelled amino acids. Since brain contains millimolar concentrations of the amino acids studied, exchange could account for the high tissue/medium ratios of radioactivity obtained. This possibility was underestimated in previous studies (Iversen & Neal, 1968; Levi, Bertollini, Chen & Raiteri, 1974).

Purified synaptosomes prepared from adult Wistar rat cerebrum (Gray & Whittaker, 1962) were prelabelled by incubating them for 10 min in a Krebs-Ringer medium containing 0.5 μM [^{3}H]-GABA and 0.1 mM aminooxyacetic acid. Spontaneous release and exchange of [^{3}H]-GABA were studied by a superfusion technique which prevents re-uptake of released substrates (Raiteri, Angelini & Levi, 1974). The following results were obtained: (1) Superfusion with unlabelled GABA (1-1000 μM) enhanced the release of [^{3}H]-GABA from prelabelled synaptosomes in a concentration dependent, saturable way. A concentration of GABA as low as 1 μM doubled the efflux of [^{3}H]-GABA. (2) The calculated exchange rates obtained with 1-20 μM unlabelled GABA were similar to the initial rates of [^{3}H]-GABA high affinity uptake reported previously (Levi & Raiteri, 1973). In particular, the exchange rate at a saturating GABA concentration (1 mM) almost coincided with the apparent V*max* of the high affinity uptake system. (3) Exchange showed a high substrate specificity, similar to that described for uptake. (4) In the absence of sodium, a condition which inhibits most of the high affinity uptake of GABA, homoexchange became undetectable even at high (1 mM) GABA concentrations. (5) When synaptosomes were incubated for 10 min in a medium containing 1, 5 or 10 μM [^{3}H]-GABA, to measure uptake in the concentration range of the high affinity system, the radioactivity decreased in the medium (−49%, −46% and −36% respectively); however, GABA concentrations did not decrease (final concentrations found: 2.1 ± 0.4, 5.5 ± 0.4, and 9.6 ± 0.9 μM respectively; averages of 4-12 determinations) as one would expect if net uptake were the main phenomenon measured. (6) With glycine, exchange at low concentrations (10 and 25 μM) was detectable only in synaptosomes prepared from