Benzodiazepines potentiate GABA-dopamine stereotyped dependent gnawing in mice

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Electrophysiological and biochemical evidence suggests that the benzodiazepines facilitate GABA-activity (Costa et al 1975; Haefely et al 1975; Biggio et al 1977; Kozhechkin & Ostrovskaya 1977; Wolf & Haas 1977); No direct facilitation of GABA was found by others although the compounds were effective in reversing the blocking action of GABA-antagonists on GABA-mediated inhibitory effects (Dray & Bowery 1978). In receptor affinity binding studies the benzodiazepines do not displace the GABA-antagonist [^{3}H]bicuculline (Möhler & Okada 1977a). However, the benzodiazepines bind specifically with high affinity to receptors in the central nervous system (Braestrup et al 1977; Möhler & Okada, 1977b; Squires & Braestrup 1977). Recently we found that various GABA-ergic drugs, including the agonist muscimol (Biggio et al 1977; Scheel-Krüger et al 1978), characteristically influenced the behavioural stimulation in mice induced by dopaminergic drugs: the stereotyped behaviour is strongly increased whereas the locomotor stimulation is antagonized (Scheel-Krüger et al 1978).

In the present study a series of benzodiazepines were tested on the dopamine-GABA-dependent stereotyped gnawing behaviour in mice. We demonstrate that the drugs in low doses potentiate the effect of muscimol on methylphenidate-induced stereotyped gnawing. The rank order corresponds to their comparative affinity for the specific benzodiazepine receptor (Braestrup et al 1977; Squires & Braestrup 1977). The benzodiazepines per se induced only a moderate increase of methylphenidate induced gnawing, reaching only 20–50% of maximum score, even after extremely high doses. In contrast to muscimol the benzodiazepines increased the methylphenidate-induced locomotor activity.

Methylphenidate, 10 mg kg^{-1} was selected (Pedersen & Christensen 1972) for this study, since this dose induces no gnawing activity (n = 50). The threshold dose for gnawing was 20 mg kg^{-1} and maximal effect was observed after 40 mg kg^{-1}. Pretreatment with muscimol HBr (0·63 – 2·0 mg kg^{-1}, s.c.) induced stereotyped gnawing after injection of methylphenidate (Fig. 1). Muscimol alone induces no stereotyped behaviour. In combined treatments the best effect was found after muscimol 1 mg kg^{-1} (62% of maximum possible score), an effect which corresponded to 30–35 mg kg^{-1} methylphenidate. Doses of muscimol higher than 1·5 mg kg^{-1} induced an increasing degree of sedation which excluded the ability for gnawing activity of the corrugated paper. Gnawing movements were still present in these mice. When given alone muscimol 2-5 mg kg^{-1} induced a state of sedation, catalepsy and loss of the righting reflex (5 mg kg^{-1}). The pretreatment with benzodiazepines given intraperitoneally induced a moderate but significant stereotyped gnawing activity after methylphenidate (10 mg kg^{-1}). The benzodiazepines in Table 1 have been tested over a wide dose range. In general the highest dose for each drug tested corresponds to 5–8 times the doses shown in Table 1. According to the drug used, the gnawing activity measured after methylphenidate reached between 20 and 50% of maximum score. No further increase was observed even

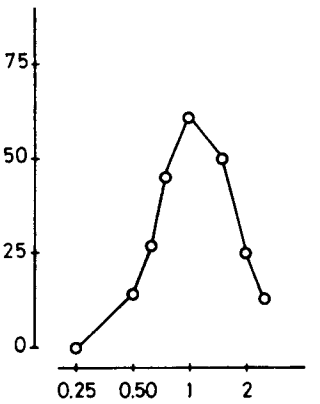

FIG. 1. Dose-response curve for muscimol-methylphenidate-induced stereotyped gnawing in mice. Muscimol HBr (abscissa) was given subcutaneously in increasing doses 15 min before methylphenidate (10 mg kg^{-1}, s.c.). Immediately after methylphenidate the mice were placed for 1 h in the "gnawing cages", 2 mice in each. The cages consisted of 30 cm high Perspex boxes (12 × 25 cm) without bottom or lid. The cages were placed on corrugated paper. For each dose of muscimol at least 10–20 pairs of mice were used. The degree of gnawing activity seen on the corrugated paper was rated according to a 4-point scale: 0, 1, 2 and 3 points. The scores obtained for gnawing activity shown on the figure are presented as percentages of the maximum possible score (ordinate).

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after extremely high doses of the benzodiazepines. Fig. 2A, B shows the dose response curves obtained for diazepam, chlordiazepoxide, clonazepam and nitrazepam. For other drugs we found the most effective doses for inducing 40–50% of maximum gnawing score were (mg kg^{-1}): lorazepam, 1; estazolam and flurazepam, 2·5; chlorazepate and medazepam, 40. Flunitrazepam (0·1–2·5 mg kg^{-1}) and nitrazepam (0·25–10 mg kg^{-1}) induced the weakest gnawing response after methylphenidate reaching only 20% of maximum possible score. That these effects are caused by an interaction between benzodiazepines and methylphenidate transport or metabolism cannot be excluded. However, this is unlikely since diazepam also induced gnawing after combination with cocaine, another dopaminergic stimulant (unpublished results). Furthermore, no evident changes in the time-course of action of methylphenidate were observed.

All the benzodiazepines, except lorazepam, in combination with methylphenidate induced a characteristic and compulsive hypermotility in the mice even after high ataxic doses (Christensen, Scheel-Krüger & Arnt, in preparation).

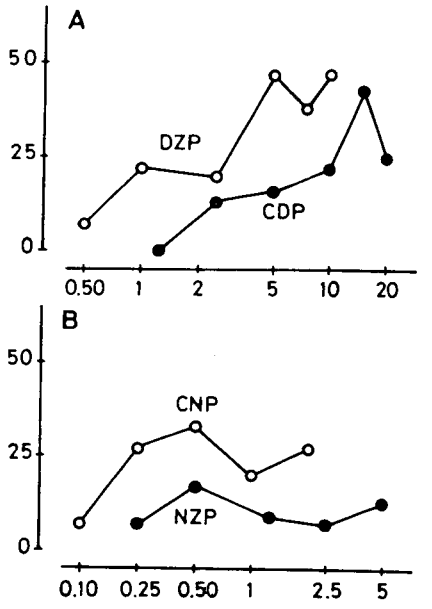

FIG. 2. Dose-response curves for A: diazepam (DZP), chlordiazepoxide (CDP), B: clonazepam (CNP), and nitrazepam (NZP) for methylphenidate-induced stereotyped gnawing. The benzodiazepines were injected intraperitoneally in various doses 15 min before methylphenidate (10 mg kg^{-1}, s.c.). Immediately after methylphenidate the mice were placed in the gnawing cages for 1 h. The gnawing activity was evaluated as stated in Fig. 1. For each dose of the benzodiazepines at least 5–10 pairs of mice have been used. Ordinate: Gnawing scores % of maximum. Abscissa: Dose (mg kg^{-1}).

Table 1. Benzodiazepines potentiate methylphenidate-muscimol gnawing behaviour in mice. The doses of the benzodiazepines in the table indicate the minimal effective doses which given in combination with muscimol (0·50 mg kg^{-1}) and methylphenidate (10 mg kg^{-1}) induce maximum possible gnawing score (data shown as % of max. ± s.e.m.). A 50–60% reduction in the doses of the benzodiazepines in this combined treatment induced a gnawing response of only 50% or less than the maximum possible. The benzodiazepines were given intraperitoneally simultaneously with the saline or muscimol (0·25 or 0·50 mg kg^{-1}, subcutaneously) 15 min before methylphenidate. Immediately after methylphenidate the mice were placed for 1 h in the "gnawing cages", two mice in each cage. The gnawing activity shown on the corrugated paper was evaluated as stated in Fig. 1. For each drug or drug combination at least 5 pairs of mice were used.

		Muscimol, mg kg^{-1}		
		0	0·25	0·50
Benzodiazepines	Doses mg kg^{-1}	Gnawing activity in % of maximum possible		
Saline		0	0	11 ± 4
Clonazepam	0·25	27 ± 8	53 ± 17	100
Lorazepam	0·25	3 ± 3	40 ± 12	100
Flunitrazepam	0·25	0	20 ± 13	93 ± 7
Diazepam	1	22 ± 6	67 ± 11	100
Estazolam	1	17 ± 3	47 ± 13	73 ± 12
Nitrazepam	1·25	10 ± 7	47 ± 13	80 ± 8
Flurazepam	1·25	10 ± 9	47 ± 13	60 ± 19
Chlordiazepoxide	5	16 ± 6	13 ± 7	83 ± 7
Chlorazepate	10	6 ± 4	20 ± 8	87 ± 13
Medazepam	10	3 ± 3	0	93 ± 7

The benzodiazepines potentiated the muscimol and methylphenidate-induced gnawing. In Table 1 are summarized the minimal effective doses of the benzodiazepines which in combination with an almost non-effective dose of muscimol (0·50 mg kg^{-1}, Fig. 1) and methylphenidate (10 mg kg^{-1}) induced the maximum possible gnawing score. The dose of muscimol used in the combined treatment was found important for induction of gnawing activity. Muscimol, 0·25 mg kg^{-1}, still induced gnawing (Table 1), whereas muscimol 0·10 mg kg^{-1} or lower doses did not increase the gnawing induced by methylphenidate and diazepam (1 and 5 mg kg^{-1} tested, n = 10 groups of mice).

The results with methylphenidate demonstrate that benzodiazepines increase some behavioural effects dependent on dopaminergic mechanisms (Pedersen & Christensen 1972; Scheel-Krüger et al 1977, 1978): the locomotor activity and stereotyped gnawing behaviour. This finding is in agreement with earlier studies (Babbini et al 1971; Rushton et al 1973; Soubrie et al 1975; D'Mello & Stolerman 1977). Enhancement of dopamin-

ergic stereotyped gnawing does not specifically indicate an involvement of GABA, since this behaviour can also be influenced by other neurotransmitters (Braestrup 1977; Scheel-Krüger et al 1977). Anti-acetylcholine drugs in particular are known to increase dopamine dependent stereotypy (Scheel-Krüger 1970). However, distinct differences exist between benzodiazepines and antiacetylcholine drugs (Costa et al 1975; Zsilla et al 1976; Ghoneim & Mewaldt 1977). In addition, diazepam counteracts the hypermotility in mice induced by such drugs (Soubrie et al 1976; Ahtee & Schillito 1970).

Although our results do not clarify the mechanism of action, the results with dopamine-dependent stereotyped gnawing behaviour are consistent with the hypothesis that the benzodiazepines may act by sensitizing GABA receptors to the action of GABA (Guidotti et al 1978). Furthermore, the potentiation of methylphenidate-induced gnawing activity seen after combined treatment with the benzodiazepines and the GABA agonist muscimol strongly suggests an increase in GABA receptor activity. This effect of the benzodiazepines is related to the potency of these drugs in some pharmacological tests (Randall et al 1974) and the rank order also corresponds closely to their comparative affinity for benzodiazepine receptors in rat and human brain (Braestrup et al 1977; Möhler & Okada 1977b; Squires & Braestrup 1977). However, this hypothesis does not explain the marked increase in locomotor activity seen after combined treatment with benzodiazepines and various "dopaminergic" stimulants, i.e. amphetamine, cocaine and methylphenidate (Rushton et al 1973; Soubrie et al 1975; D'Mello & Stolerman 1977) which contrast with the marked antagonism of this hyperactivity by muscimol and other GABA-ergic drugs (Scheel-Krüger et al 1978). The results with motility emphasize that not all actions of the benzodiazepines are related to a facilitation of GABA neurotransmission.

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