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BEHAVIOURAL AND PHARMACOKINETIC STUDIES IN THE MONKEY (*Macaca mulatta*) WITH DIAZEPAM, NORDIAZEPAM AND RELATED 1,4-BENZODIAZEPINES

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1 Behavioural activity (delayed differentiation and spatial delayed alternation) and pharmacokinetics of diazepam and its metabolites, *N*-desmethyldiazepam (nordiazepam), 3-hydroxydiazepam (temazepam) and 3-hydroxy-*N*-desmethyldiazepam (oxazepam), and of dipotassium clorazepate (clorazepate), were studied in the monkey (*Macaca mulatta*). Diazepam and its metabolites (1.8 and 3.0 mg/kg) and clorazepate (2.6 and 4.3 mg/kg) were given by intraperitoneal injection.

2 Hydroxylation of diazepam (temazepam and oxazepam) led to a loss of, or a considerable reduction in, behavioural activity, whereas activity was preserved, though modified, by demethylation (nordiazepam). It was not possible to establish change in behaviour at specific time intervals after clorazepate, but combined performance data revealed an effect.

3 The maximum mean plasma concentrations of diazepam, temazepam, oxazepam and clorazepate were observed at 0.5 h, and the maximum mean plasma concentration of nordiazepam was observed at 1 hour. Plasma concentrations of nordiazepam were the highest and decreased monoexponentially. Plasma concentrations of the other drugs declined rapidly at first but more slowly later, and these data were analysed as biexponential models. In the analysis for metabolites, nordiazepam reached measurable levels after the injection of diazepam and clorazepate.

4 It is suggested that differences in the effects of closely related benzodiazepines may not be due solely to their plasma pharmacokinetic properties, but may arise from differences in their intrinsic activity.

Introduction

In previous studies we have been concerned with the effect of barbiturates and benzodiazepines on delayed differentiation behaviour in the monkey (*Macaca mulatta*). With barbiturates and benzodiazepines behaviour is impaired (Nicholson, Wright & Ferres, 1973; Nicholson & Wright, 1974) but the effect of benzodiazepines would appear to be more complex than that of the barbiturates. Unlike the barbiturates, impaired differentiation with benzodiazepines may be related to the nature of the response demanded (GO or NO–GO), and this would suggest that inhibitory and disinhibitory behaviour may be involved.

In the present study we have investigated the behavioural and pharmacokinetic activity of diazepam and its metabolites. Minor alterations to the diazepam molecule, demethylation or hydroxylation (Figure 1), may be associated with specific changes in behavioural effect, and so suggest relations between molecular configuration and activity. It is in this context that we have studied the activity of the metabolites of diazepam, as well as a precursor of nordiazepam, dipotassium clorazepate (clorazepate).

Methods

Behavioural studies

A colony of 5 male monkeys (*Macaca mulatta*) was trained on delayed differentiation. The task (Konorski, 1959; Roberts & Bradley, 1967; Nicholson *et al.*, 1973) required the animal to differentiate between two stimuli separated by a 4 s delay. The animal was required to press a lever during the presentation of the second stimulus if the stimuli were like (GO response), and to refrain from pressing the lever if the stimuli were unlike (NO-GO response). Correct GO and NO-GO responses were rewarded with food. Each

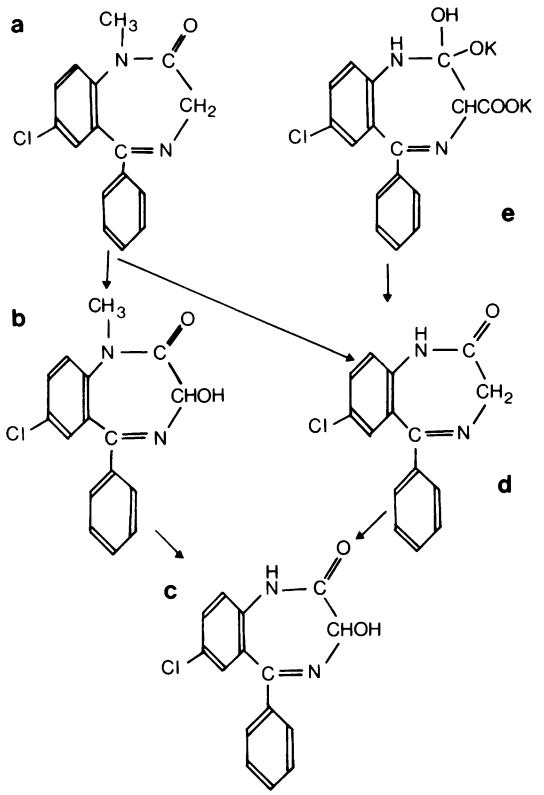

Figure 1 Structural formulae of (a) diazepam, (b) 3-hydroxydiazepam (temazepam), (c) 3-hydroxy-*N*-desmethyldiazepam (oxazepam), (d) *N*-desmethyldiazepam (nordiazepam) and (e) dipotassium clorazepate (clorazepate).

experiment consisted of 8 sessions of 50 trials. Two sessions were held each day at approximately 11 h 00 min and 15 h 00 min. Days 1 and 2 each consisted of two control sessions. On day 3 the drug was given at approximately 09 h 00 min and testing sessions were held exactly 2 and 6 h later. Day 4 consisted of 2 sessions held at approximately 11 h 00 min and 15 h 00 min. The second session of each day was held 4 h after the first session of that day. At least 7 days separated each injection. Total response time for GO responses (from onset of the second stimulus to response) and the correctness of each response were recorded.

Another colony of 5 male monkeys (*Macaca mulatta*) was trained on spatial delayed alternation using a re-run correction method. The stimuli were two illuminated green circles displayed simultaneously on two vertical panels spaced apart. A central food well was situated below the panels, and the animal was required to alternate responses between right and left panels. Each experiment consisted of 51 trials. With the initial presentation of stimuli the monkey pressed one of the green circles to extinguish the stimuli. The animal was rewarded with a food pellet, but the first response was not used in the analysis of performance. With succeeding trials correct performance was a response to the panel not rewarded on the previous trial. The delay between trials was 5 s, and each monkey was required to reach a level of performance of 44 correct out of 50 responses for 10 consecutive sessions (criterion). Each animal was tested 2 h after injection of drug or placebo but during the course of the experiments the pharmacokinetic data became available and so additional experiments were carried out in which performance was tested 1 h after 3.0 mg/kg temazepam and oxazepam, and 4 and 6 h after 3.0 mg/kg diazepam and nordiazepam. The order of the treatments was randomized for each monkey, and at least 4 days separated each injection.

In both behavioural studies diazepam, temazepam, nordiazepam and oxazepam were injected intraperitoneally at doses of 1.8 and 3.0 mg/kg body weight in 5 ml polyethylene glycol. In the differentiation experiments clorazepate was studied at 2.6 and 4.3 mg/kg body weight in 5 ml buffered 0.9% w/v NaCl solution (saline, pH 7.8). In both experiments the placebo was the drug vehicle, and analysis of variance was used as the statistical procedure.

Pharmacokinetic studies

Another colony of male monkeys (*Macaca mulatta*) weighing between 5.4 and 6.2 (mean 5.8) kg was used. Six of the animals were injected intraperitoneally on separate occasions with 3.0 mg/kg diazepam, nordiazepam, temazepam and oxazepam, and 4.5 mg/kg clorazepate. Blood samples were obtained at 0.5, 1, 2, 4, 8 and 24 h after injection. Accepted methods for the gas-liquid chromatographic separation and determination of benzodiazepines were used (De Silva & Puglisi, 1970).

Results

Behavioural studies

Analysis of delayed differentiation involved change in total response time for GO responses, and change in accuracy of response to matching (excluding repeated responses if the initial trial was incorrect). The measures were related to the performance of individual monkeys, dose level and time after injection. With total response time it was not possible to separate effects of dose, and effects were limited to 2 h after injection (Table 1). Total response time was increased with diazepam ($P < 0.01$) and nordiazepam ($P < 0.05$), but no effect was observed with temazepam and oxazepam. With clorazepate the change in total

Table 1 Change of total response time (ms) after drugs from that after placebo for correct GO responses (delayed differentiation)

Time after injection (h)	*Diazepam*	*Nordiazepam*	*Temazepam*	*Oxazepam*	*Clorazepate*
2	93.5	80.6	4.9	20.9	56.9
	**	*	NS	NS	NS
6	40.4	51.1	−0.4	−7.3	41.3
	NS	NS	NS	NS	NS

Values are mean for 5 monkeys. NS: not significant.
Least significant differences

*$P<0.05$	**$P<0.01$	***$P<0.001$
65.2	89.9	100.1

Table 2 Change of number of correct responses 2 h after drugs from that after placebo for GO and NO-GO responses (delayed differntiation)

Response analysed	*Diazepam*	*Nordiazepam*	*Temazepam*	*Oxazepam*	*Clorazepate*
All responses	−1.5	−2.6	0.9	−0.4	−0.8
	NS	**	NS	NS	NS
GO	0.6	−1.2	0.4	−0.1	0.2
	NS	*	NS	NS	NS
NO-GO	−2.2	−1.3	0.1	−0.4	−0.9
	***	*	NS	NS	NS

Values are mean for 5 monkeys. NS: not significant.
Least significant differences

*$P<0.05$	**$P<0.01$	***$P<0.001$
All 1.90	2.62	2.92
GO 1.19	1.64	1.82
NO-GO 1.03	1.42	1.58

Table 3 Number of correct responses out of 50 (spatial delayed alternation) after drugs

Time (h) after injection	*Diazepam (mg/kg)*		*Nordiazepam (mg/kg)*		*Temazepam (mg/kg)*		*Oxazepam (mg/kg)*	
	1.8	*3.0*	*1.8*	*3.0*	*1.8*	*3.0*	*1.8*	*3.0*
1						46.4		45.6
						NS		NS
2	41.4	41.6	46.0	42.6	45.6	46.4	46.6	47.0
	**	**	NS	*	NS	NS	NS	NS
4		45.6		47.6				
		NS		NS				
6		46.4		45.6				
		NS		NS				

Values are mean for 5 monkeys. Mean placebo value = 46.9. NS: not significant.
Least significant differences

*$P<0.05$	**$P<0.01$	***$P<0.001$
42.9	41.7	40.1

response time 2 h after injection was not significant, but when the 2 and 6 h data were combined the increase in total response time was significant ($P < 0.05$).

With accuracy of response to matching it was not possible to separate the effect of dose. Effects were limited to 2 h after injection (Table 2). Overall accuracy (all responses) was impaired with nordiazepam ($P < 0.01$), and was due to impaired responses to both GO and NO-GO situations ($P < 0.05$). With diazepam impaired accuracy of response to matching was due to incorrect responses to the NO-GO situation only ($P < 0.001$). Accuracy of response to matching was not impaired with temazepam, oxazepam and clorazepate.

Analysis of alternation behaviour involved the number of correct responses for all monkeys for all treatments (drug and placebo), and for each time interval after injection (Table 3). It was possible to separate effects related to dose. There were no significant differences between the placebo values related to the separate studies, and so the values were combined to give a single mean placebo value (46.9 correct responses). At 2 h after injection, alternation performance was impaired by both doses of diazepam ($P < 0.01$), but with nordiazepam only at the 3.0 mg/kg dose ($P < 0.05$). No effects on alternation performance were observed at 4 and 6 h after 3.0 mg/kg diazepam and nordiazepam or at 1 and 2 h after 3.0 mg/kg temazepam and oxazepam.

Pharmacokinetic studies

The mean concentration of nordiazepam was the highest. The peak concentration was observed at 1 h, and declined monoexponentially. It was likely that the equilibration phase was absent, so that the drug was rapidly distributed throughout the body water with little tissue binding. Plasma concentrations of the other drugs declined rapidly at first, but more slowly later, and these data were analysed as biexponential models using a method of residuals (Riggs, 1970). There was some evidence of a three phase fall with oxazepam, but insufficient data were available to confirm this impression. The rapid falls in the plasma concentrations of temazepam and oxazepam suggested fast tissue penetration, but the similar falls with diazepam and clorazepate were more likely to be related to their conversion to nordiazepam, as two phases can occur if separate metabolic reactions, one with a rate constant much greater than the other, exist together. In the analysis for metabolites, nordiazepam reached measurable levels after injection of diazepam and clorazepate (Tables 4 and 5, Figure 2).

Discussion

Though the effect of the parent compounds, diazepam and clorazepate, are uncertain because of the activity of their common metabolite, nordiazepam, the activity

Table 4 Mean plasma concentrations (μg/ml) in the monkey (*Macaca mulatta*) after intraperitoneal injection

Compound		*Time (h)* 0.5	1	2	4	8	24
Unmetabolized drugs							
Clorazepate	Mean	1.81	1.10	0.80	0.30	0.05	0.02
	s.e.	0.70	0.31	0.20	0.11	0.02	0.01
Diazepam	Mean	0.55	0.33	0.11	0.04	0.03	0.00
	s.e.	0.13	0.07	0.02	0.01	0.01	–
Nordiazepam	Mean	2.61	2.72	2.11	1.84	1.40	0.35
	s.e.	0.67	0.38	0.29	0.24	0.21	0.07
Temazepam	Mean	0.71	0.46	0.17	0.04	0.02	0.00
	s.e.	0.23	0.13	0.05	0.01	–	–
Oxazepam	Mean	1.38	0.87	0.39	0.22	0.05	0.01
	s.e.	0.42	0.19	0.13	0.06	0.02	0.01
Nordiazepam as a metabolite of other drugs							
After clorazepate	Mean	0.99	1.16	1.08	0.57	0.51	0.09
	s.e.	0.19	0.37	0.27	0.09	0.11	0.01
After diazepam	Mean	1.77	2.05	1.57	1.27	1.06	0.25
	s.e.	0.39.	0.38	0.20	0.21	0.13	0.05

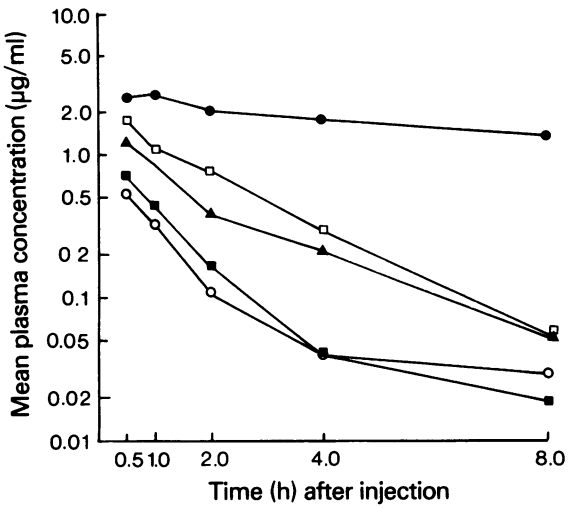

Figure 2 Mean plasma concentrations (µg/ml) of 3.0 mg/kg diazepam (○), nordiazepam (●), temazepam (■), oxazepam (▲) and 4.5 mg/kg clorazepate (□) after intraperitoneal injection.

of nordiazepam, temazepam and oxazepam are not complicated in this way. Within similar dose ranges temazepam and oxazepam are without effects on delayed differentiation and spatial delayed alternation in the monkey, whereas nordiazepam leads to changes, though different from those observed with diazepam. However, such differential effects are not obvious in studies on behaviour with rodents. Diazepam and its three metabolites may differ in their relative potencies, but each possesses anticonvulsant and muscle relaxant activity, potentiates hexobarbitone sleeping time and the effect of tetrabenazine and increases spontaneous motor activity (Randall, Scheckel & Pool, 1970; Marcucci, Mussini, Guaitani, Famelli & Garattini, 1971; De Angelis, Traversa & Vertua, 1974; Babbini, Torielli, Gaiardi, Bartoletti & De Marchi, 1974; Babbini, Gaiardi, Bartoletti, Torielli & De Marchi, 1975). Closely related benzodiazepines may have similar effects in rodents, but it would appear that their effects, as revealed by more complex analyses in the monkey, may differ, and that these differences do not depend solely on pharmacokinetic considerations.

Studies in man support the possibility of differential effects of benzodiazepines on the central nervous system. Diazepam and its three metabolites each possess hypnotic activity within the same dose range, but their effects on sleep differ. Pharmacokinetics may

Table 5 Early and late phase half times (h) during the first 24 h after intraperitoneal injection

	$T_{\frac{1}{2}}$
Diazepam	0.50 and 9.69
Nordiazepam	8.15 only
Temazepam	0.60 and 4.56
Oxazepam	0.57 and 10.20
Clorazepate	0.60 and 11.0

Values are mean for 6 monkeys.

play a part, as changes in sleep onset latency and persistence of effect may be related to absorption and elimination, but, whereas diazepam has little or no effect on drowsy (stage 1) sleep, all three of its metabolites markedly reduce the duration of this stage. Further, latency to stage 3 sleep is shortened by nordiazepam and latency to the first REM episode is delayed by temazepam, whereas these effects are not observed with the other metabolites of diazepam (Nicholson & Stone, 1976, and unpublished results; Nicholson, Stone & Clarke, 1976; Nicholson, Stone, Clarke & Ferres, 1976). There are also differential effects on performance. Diazepam, temazepam and oxazepam impair psychomotor performance in man, whereas with nordiazepam impaired psychomotor activity may not be observed, though the ability to maintain a high level of performance over several hours may be affected (Palva & Linnoila, 1976; Clarke & Nicholson, unpublished observation).

The present observations in the monkey may be relevant to the use of these drugs. It is considered that the studies do not relate to their anxiolytic or hypnotic properties, but to more subtle, though as yet uncertain, effects on higher nervous function. Differential effects of closely related benzodiazepines may not be easily demonstrated in rodents, but may be revealed by behavioural studies in primates. The significance to man of the observations in the monkey is not clear, but, together with the studies on the effect of these drugs on sleep and psychomotor performance, they suggest that higher nervous function in man may be modified in different ways by separate benzodiazepines.

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