CHANGES IN REACTION TIME AND DRUG PLASMA CONCENTRATIONS AFTER NITRAZEPAM AND GLUTETHIMIDE

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- 1 The effects of nitrazepam, glutethimide and placebo were assessed in a double-blind cross-over study with five healthy human subjects, using reaction time, and relating changes to drug concentrations in the plasma.
- 2 After glutethimide in all five subjects, and after nitrazepam in four subjects, larger doses led to higher concentrations in plasma and to greater changes in reaction time; one subject displayed the higher nitrazepam concentrations and the greater effect following the smaller dose.
- 3 The relationship between change in reaction time and concentration appeared to be mainly a within-subject dose-effect relationship, with the peak concentration in plasma as the 'dose' and the area under the reaction time curve as the effect.

Introduction

Recent research in clinical psychopharmacology has been much concerned with: (i) techniques for the quantitation of effects of centrally-acting drugs; and (ii) concentrations of drug molecules in plasma. However, studies of behaviour and pharmacokinetic factors together are relatively few. A number of different types of observation have been recorded when these two aspects of the pharmacology of drugs with CNS-depressant activity have been studied synchronously. For example: (i) autonomic, but not behavioural, effects of chlorpromazine followed closely the concentrations of unmetabolized drug in plasma (Sakalis, Lader, Mould & Curry, 1972); (ii) effects of glutethimide and ethanol, given together, related closely to concentrations of the two drugs in plasma (Mould, Binns & Curry, 1972); (iii) peak effects of ethinamate appeared to lag 15 min behind peak plasma levels (Speirs, Virden & Scott, 1973); (iv) drowsiness occurred most frequently in subjects with high plasma concentrations of diazepam (Garattini, 1969); (v) alleviation of anxiety during diazepam treatment related to concentrations of the metabolite N-desmethyldiazepam (Robin, Curry & Whelpton, 1974); (vi) peak effects of glutethimide on smooth tracking eye movement preceded peak concentrations in

plasma (Curry & Norris, 1970); (vii) some effects of ethanol were greater during ethanol absorption, than when absorption had virtually ceased, resulting from uneven distribution of ethanol through body fluids during absorption (Jones & Vega, 1972; Haggard & Greenberg, 1934).

There appear to have been no studies in this field with nitrazepam, and we now report a comparative assessment of nitrazepam and glutethimide, with pharmacological assessment using reaction time.

Methods

The subjects were five healthy adult volunteer medical students, one female and four males whose ages ranged from 19 to 21 years. They were given five treatments allocated by means of latin square design, nitrazepam 5 and 10 mg, glutethimide 250 and 500 mg, and placebo, in matching capsules on separate occasions, one week apart. The study was double-blind and the code was held by a colleague who had no direct contact with the subject or experimenters.

The subject, fasting 4 h and having not smoked or taken alcohol, presented himself/herself for the

study, always in the afternoon. With the subject sitting comfortably in an armchair in a darkened room, an electronic counter registering ms was used to obtain reaction times. It registered the delay between a flash which started the counter and the subject pressing a switch which stopped the counter. This observation was repeated ten times and the average of these values represented the reaction time for that particular point. After the reaction times had been obtained a venepuncture was taken. The procedure was repeated on five occasions at half hourly intervals. Plasma was separated by centrifugation of the blood collected at each time except the 2 h point into heparinized tubes.

Assay of nitrazepam

The method utilized hydrolysis of nitrazepam to 2-amino-5-nitro-benzophenone (ANB). To a solution of nitrazepam in water or plasma (both

synthetic standards and experimental samples) (1 ml) was added to 0.1N Na_2HPO_4 (1 ml) and toluene (6 ml). After shaking (15 min) and centrifugation, a toluene aliquot (5 ml) was transferred to a tube containing 2N HCl (2.5 ml). After shaking (15 mins) and centrifugation, 2 ml of the acid layer were removed to a clean tube and boiled for 1 h. Ammonia (0.88) (0.5 ml) was added, and the ANB was extracted into 0.2 ml of toluene containing 15% amyl alcohol. The organic layer was collected in the bottom of a tapered centrifuge tube by means of a series of aspiration and centrifugation steps. Samples (3 μl) were injected into a Pye model 104 gas chromatograph, equipped with a Ni-63 electron capture detector, operated in the pulsed mode with a pulse space of 150 μs . The detector oven was at 300°C. The column containing 5% OV-17 on chromosorb W HP, 80-100 mesh, 9 ft, was maintained at 260°C. The carrier was nitrogen 50 psi (flow rate 60 ml/min). Direct on-column injection was

Table 1 Reaction times at various times after five treatments in five subjects

<i>Treatment</i>	<i>Subject number</i>	<i>Order of treatments</i>	<i>Reaction time (ms) at various times (h after dose)</i>					
			<i>0</i>	<i>0.5</i>	<i>1</i>	<i>1.5</i>	<i>2</i>	<i>2.5</i>
Placebo	1	2	145	141	150	145	141	150
	2	3	227	220	202	227	220	202
	3	1	187	185	203	184	185	203
	4	4	144	168	174	144	168	174
	5	5	181	180	157	181	180	157
	Mean	—	176.8	178.8	177.2	176.2	178.8	177.2
Nitrazepam (5 mg)	1	5	162	160	160	184	168	191
	2	2	206	192	209	203	188	187
	3	3	174	195	193	186	188	210
	4	1	175	166	168	201	180	211
	5	4	162	182	194	176	186	189
	Mean	—	175.8	179.0	184.8	190.0	182.0	197.6
Nitrazepam (10 mg)	1	4	138	148	154	182	179	165
	2	5	173	234	223	247	224	221
	3	2	193	207	220	223	208	190
	4	3	152	168	179	244	218	185
	5	1	219	219	204	196	195	200
	Mean	—	175.0	195.2	196.0	218.4	204.8	192.2
Glutethimide (250 mg)	1	1	164	160	164	167	167	167
	2	4	181	196	192	193	197	232
	3	5	176	203	198	197	213	195
	4	2	153	168	159	187	174	145
	5	3	167	182	183	192	198	193
	Mean	—	168.2	181.8	179.2	187.2	189.2	186.4
Glutethimide (500 mg)	1	3	150	179	167	159	153	162
	2	1	193	215	249	255	229	227
	3	4	173	275	209	195	188	188
	4	5	168	156	206	227	234	164
	5	2	182	175	183	183	184	165
	Mean	—	173.2	200.0	202.8	203.8	197.6	181.2

employed. Comparison of experimental peaks (retention time 5 min) with those in extracts from synthetic solutions of nitrazepam in blank plasma was by measurement of peak heights. The peaks corresponded in properties with that from an authentic sample of ANB. Recovery was $97 \pm 2.0\%$ (s.e. mean, $n = 5$) from samples containing $0.1 \mu\text{g}$ and $102 \pm 2.6\%$ (s.e. mean, $n = 5$) from samples containing $0.05 \mu\text{g}$.

Assay of glutethimide

This was carried out by a method previously reported (Grieveson & Gordon, 1969; Curry, Riddall, Gordon, Simpson, Binns, Rondel & McMartin, 1971).

Results

Results are presented as raw data for reaction times and concentrations of the two drugs (Tables 1, 2 and 3), and as a diagram of mean changes from pretreatment reaction times (Figure 1). All pretreatment samples and samples following placebo administration were devoid of drug content.

Reaction times

Mean reaction time after placebo was virtually unchanged over the 2.5 h time period. In contrast, all four active doses caused an increase in mean reaction time, the greatest difference being a mean increase of 43.4 ms (24.8%) after nitrazepam

Figure 1 Changes in mean reaction time (ms) in five subjects given five treatments (● placebo; ■ glutethimide 500 mg; □ glutethimide 250 mg; ▼ nitrazepam 10 mg; ▽ nitrazepam 5 mg).

(10 mg) at 1.5 h.

The set of five pretreatment scores from each individual obtained over the 5 week period provided data for assessment of reproducibility of reaction time measurements. No particular trend was apparent in any individual, but mean scores on the five test occasions (in order) were: 187.6, 175.8, 174.0, 159.8 and 172.0. There was thus a suggestion of a practice effect from these data considered in isolation. The existence of a small practice effect was borne out by analysis of variance using the data for the entire experiment (Table 4). There was a high degree of between-

Table 2 Concentrations of nitrazepam in five subjects following two doses

Treatment	Subject number	Concentration (ng/ml) at various times (h) after dose			
		0.5	1	1.5	2.5
Nitrazepam (5 mg)	1	4	20	34	43
	2	10	27	32	—*
	3	39	50	55	38
	4	8	13	28	34
	5	8	18	38	53
	Mean	13.8	21.3	37.4	42.0
Nitrazepam (10 mg)	1	9	53	53	43
	2	35	78	75	85
	3	13	50	50	83
	4	3	18	41	3
	5	3	9	3	24
	Mean	12.6	40.2	44.4	47.6

* No Sample obtained

subject variation in the data ($P < 0.001$). The variation between treatments was also significant ($P < 0.01$) as was the variation between times ($P < 0.001$). Specific differences from placebo in mean reaction times were detected by means of two-tailed *t*-tests for related samples as follows: nitrazepam at 1.5 (10 mg) and 2 h (5 and 10 mg) ($P < 0.05$); glutethimide (250 and 500 mg) at 1 h ($P < 0.02$). Additionally, the differences between the scores following the high and low nitrazepam doses at 1.5 ($P < 0.05$) and 2 h ($P < 0.02$) were significant.

Considering the reaction times for each subject individually, number 1 showed little change after placebo and the greatest change following nitrazepam (10 mg.) Subject number 2 showed an analogous pattern, but with similar increases following the higher doses of both drugs. Subjects 3 and 4 showed increases after placebo, but greater increases following all four active doses. Subject 5 was unusual, in that no reading after nitrazepam (10 mg) was greater than the pretreatment reading,

although there was an increase in reaction time at the 2.5 h point. However, there were increases after nitrazepam (5 mg) and glutethimide (250 mg), and a very small increase after glutethimide (500 mg).

Drug concentrations

The drugs reached their highest recorded concentrations in plasma at 1, 1.5 or 2.5 h, dependent on subject and dose. Mean concentrations were highest at 2.5 h (nitrazepam), 1 and 1.5 h (glutethimide (250 mg) and 1.5 h (glutethimide 500 mg).

Analysis of variance applied to the drug concentration data was considered unnecessary. Apart from obvious differences from pretreatment and placebo readings, the following differences were found to be significantly different from each other by *t*-testing ($P < 0.05$): readings after nitrazepam (5 mg) at 0.5, 1 and 1.5 h. Note that the 1.5/2.5 h comparison was not significant. Similarly, readings

Table 3 Concentrations of glutethimide in five subjects following two doses

Treatment	Subject number	Concentration ($\mu\text{g/ml}$) at various times (h) after dose			
		0.5	1	1.5	2.5
Glutethimide (250 mg)	1	2.6	3.0	2.6	2.3
	2	1.1	3.1	2.5	2.3
	3	0.2	0.4	1.1	1.5
	4	0.7	1.5	1.9	1.4
	5	1.0	2.2	2.1	2.0
	Mean	1.1	2.0	2.0	1.9
Glutethimide (500 mg)	1	3.0	4.0	4.4	3.4
	2	3.8	4.7	3.6	3.5
	3	4.3	4.7	3.7	2.8
	4	0.9	2.5	3.1	2.3
	5	0.8	1.4	4.0	4.3
	Mean	2.6	3.5	3.8	3.3

Table 4 Analysis of variance of reaction time data

Source of variation	D.F.	Sum of squares	Variance	F	P
Subjects	4	45589.9	11397.5	36.320	< 0.001
Occasions	4	5322.5	1330.6	4.240	< 0.025
Treatments	4	7639.3	1909.8	6.086	< 0.01
Residual	12	3766.0	313.8		
Total	24				
Time	5	6352.1	1270.4	4.733	< 0.001
Treatment-time interaction	20	5991.4	299.6	1.116	NS
Residual	100	26844.4	268.4		
Total	149	101505.6			

after nitrazepam (10 mg) at 0.1 and 1 h ($P < 0.05$) were significantly different from each other. Differences between plasma concentrations after high and low doses of glutethimide were significant at all time points ($P < 0.05$) but differences after the two doses of nitrazepam were not significant. In more detail, in the twenty (five subjects at four times) possible comparisons of high and low dose glutethimide, concentrations were higher after the higher doses on eighteen occasions, but for nineteen analogous comparisons for nitrazepam (one sample was lost in the analysis) concentrations were equal or higher after the higher doses on only eleven occasions. The two exceptions with glutethimide were at early time points in Subject 5. With nitrazepam, subjects 3 (twice), 4 (twice) and 5 (all four occasions) were exceptional in showing the lower concentrations following the higher doses. However, with glutethimide in Subject 5, and with nitrazepam in Subjects 3 and 4, the highest *peak* concentrations still followed the highest doses.

Relation between plasma concentration and reaction time

Highest mean reaction times following the four drug doses coincided with, or were within one experimental point of, the highest mean drug concentration. This would appear to indicate that the two phenomena had the same time course. However, such an observation must be considered with care. When behavioural or biochemical characteristics show growth and decay patterns, and they are measured intermittently, as is the case for both phenomena in this study, displacement of times of maxima by at least two experimental points is essential in proving a lack of coincidence of the time courses of the two patterns. With a limited number of time points, the scope for demonstration of such an effect is obviously limited. However, individual data are more likely to prove useful in this regard, and in the twenty (five subjects at four times) possible comparisons of time of concentration maximum and time of greatest increase in reaction time, the two events were coincident, or within one experimental time point of each other in sixteen comparisons (including cases involving ties and including the 2.5 h time point for Subject 5 after nitrazepam (10 mg). Thus the majority of the evidence is still in favour of coincidence of the two maxima.

Phenomena showing growth and decay characteristics can also be analysed in terms of rates of growth and decay, time and height of peak levels, and areas under the curve of measurements against time. A partial analysis of this type was

attempted. Rates were not computed, as many more time points would have been needed, but it is of interest that both drugs led to the highest areas under the reaction time curves (calculated on change from pretreatment score) following the higher doses in subjects 1-4. As with the overall drug concentration data, subject 5 was exceptional, with the lower doses causing the greater effects with both drugs. The individual readings showed this relation at the majority of the time points in this subject, although the pattern was not quite so clear with reaction times as with the concentration data, presumably because reaction time data relate so much to pretreatment scores and placebo responses. In the other subjects there were nine other isolated cases of the slowing of reaction time at odd points being greatest after smaller doses, involving three subjects, but there was no pattern to these observations, which comprised a minority in a total of 120 comparisons. It is thus impossible to escape the conclusion that subject number 5 showed anomalous drug responses which related more closely to drug concentrations in plasma than to dose.

Further analysis was made by calculating individual Spearman rank correlation coefficients for reaction time and concentration in the twenty subject treatment cases. Only four of these coefficients reached significance at the 5% level. When the mean readings at the various time points after each treatment were similarly assessed none of the coefficients were significant. When the mean areas under the curves of concentration and effect for the four treatments in five subjects were calculated the correlation coefficients were again non-significant.

Finally, and as implied earlier, when high and low doses in each subject were compared, in all but one case a greater effect (from area under the curve) occurred with a greater concentration curve area. However, when the peak concentration was similarly compared with the area under the effect curve, every case showed a greater effect with a higher level. Thus the most obvious relation was a within-subject dose-effect relation based on peak concentration as the 'dose' and the area under the reaction time curve as the effect.

Discussion

When assessed in this study, at clinical dose levels, both drugs affected reaction time to similar extents, and with similar time courses, although changes occurred a little earlier with glutethimide, reflecting the earlier occurrence of peak concentrations in plasma with this drug. Nitrazepam was the

more potent of the two. The most dramatic differences were between doses and placebo, between subjects, and within the time-course of effect of each drug dose. There was a relatively small practice effect throughout the investigation.

The emphasis in this study was on simultaneous assessment of pharmacological effects and pharmacokinetic factors, and there was no evidence for a separation in time of peak effect and peak plasma levels. However, different subjects displayed different degrees of impairment at similar levels, and it appears that the relation between effect and level takes the form summarized in the following statements; (i) peak effects and levels occur

simultaneously; (ii) in each subject, higher levels lead to greater effects, but absorption can be sufficiently erratic for the anticipated dose-concentration relation to be reversed on occasions; (iii) it is incorrect to apply the word "correlation" to this relation, as when correlation statistics are applied, there is certainly no between-subject correlation, and even the changes occurring with time after one dose fail to reveal statistically significant correlations; (iv) the relation is best shown as a within-subject dose-effect relation between peak concentration in plasma and overall effect assessed by means of the area under the effect-time curve.

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