Effect of the 1,5-benzodiazepines, clobazam and triflubazam, on the sleep of man

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Many of the 1,4-benzodiazepines used as hypnotics have persistent effects on performance, but it would appear that the 1,5-benzodiazepine, clobazam, has limited residual sequelae (Caille & Bassano, 1974; Borland & Nicholson, 1975). The possibility that 1,5-benzodiazepines may possess less potential for impairing performance in man raises the question of their use as hypnotics for persons involved in skilled activity the next day. It is in this context that we have investigated the hypnotic properties of two members of this group of drugs – clobazam (1-methyl-5-phenyl-7-chloro-1H-1, 5-benzodiazepine-2, 4(3H, 5H)-dione) and triflubazam (1-methyl-5-phenyl-7-trifluoromethyl-1H-1, 5-benzodiazepine-2, 4(3H, 5H)-dione).

The subjects were six healthy male volunteers aged between 21 and 28 years. The assessment of each treatment (matching placebos or dose or a drug) involved four days. For two nights the subjects slept at home and retired at a set time, and for the next two nights they slept in the laboratory. They were requested to refrain from napping and undue exercise, and to abstain from caffeine and alcohol from mid-day on the days which involved recordings. Nine to twelve days separated each assessment. On each occasion the subject ingested two identical capsules, and the study was double blind. Preliminary studies suggested that the appropriate dose ranges to study were clobazam (10–20 mg) and triflubazam (20–40 mg).

The effect of the drugs was limited to the night of ingestion. With both drugs no statistically significant changes were observed in total sleep time or in the duration (min) of each sleep stage. With clobazam (10 & 20 mg) sleep onset latency (SOL) was reduced ($P < 0.05$), and the latency to stage 3 was shortened. There was some evidence of reduced awakenings to stage 0 and stage 1 activity, and with both 10 and clobazam (20 mg) the percentage of total sleep time occupied by stage 1 activity was less ($P < 0.01$). With clobazam (20 mg) there was an increase in the percentage of stage 2 sleep ($P < 0.05$) related to the second two hourly interval of sleep from SOL, and an overall decrease in the percentage of stage 3 ($P < 0.01$) and stage 3 + 4 ($P < 0.05$) sleep. No changes were observed in rapid eye movement sleep.

With triflubazam (20 & 40 mg) there was no change in latencies to sleep stages. There was some evidence of reduced awakenings to stage 0 and stage 1 activity. With 20 mg the percentage of total sleep time occupied by stage 1 activity was less ($P < 0.05$), but this change was not observed with the 40 mg dose. No other changes were observed.

It would appear that the hypnotic activity of the 1,5-benzodiazepines is limited, and that this is particularly so in the case of triflubazam. However, the effect of clobazam on sleep, and its restricted effects on performance (Borland & Nicholson, 1975) suggest that it may be a useful drug in the management of limited sleep difficulties in persons involved in skilled activity.

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Behavioural changes in rats suggesting drug-induced headache

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Nitroglycerin and similar organic nitrates lower blood-pressure and are liable to induce headache in man after inhalation or skin contact. If rats also get headaches, they would be expected to show: (1) Conditioned taste aversion; drugs like lithium producing unpleasant symptoms in man often cause rats to avoid novel but otherwise attractive bait (Nachman & Hartely, 1975). (2) An unspecific reduction in spontaneous behaviour (whereas strongly conditioned behaviour, e.g. to avoid shock, might overcome a mild deficit).

Twenty-four albino rats were caged in pairs, separated for 6 h each week day, and given water for 2 h daily while separated. Social behaviour was observed on return to the home cage (Silverman,

1971) classifying the acts and postures described by Grant & Mackintosh (1963) as non-social (exploration, grooming, etc.), social approach (including aggression), and escape from the other rat.

Nitroglycerin was administered as a 10% solution in corn-oil, syringing 0.5 ml/kg on to the shaved flank (Clark & Litchfield, 1967) 30 min after removing water and 15–30 min before test. Treating only one rat of each pair, nitroglycerin did not affect social behaviour. When both were treated, the total number of actions of all kinds observed in 3 min averaged 262 (±21 s.e. mean) for the six pairs of nitroglycerin-treated rats. This was a decline of 20 ± 20 actions from the pre-experimental baseline, while corn-oil controls increased by 48 ± 26 to 302 ± 24. The difference was as predicted ($t = 2.04$, $P < 0.05$ 1-tailed) but was mainly in social approach, with little change in non-social or escape behaviour.

Conditioned aversion: Instead of water, rats were offered a solution of 0.1% saccharin sodium on the day nitroglycerin was first administered. Comparing water on the previous day with saccharine on day 2 (21 h after dosing), controls increased intake by 10.2 ± 1.4 g, rats given nitroglycerin by only 7.1 ± 1.6 g ($t = 1.81$, $df = 20$, $P < 0.05$ 1-tailed).

Tolerance has been reported to nitroglycerin with supersensitivity following withdrawal, and another conditioned aversion experiment tested this.

On days 1–5 rats were offered 0.1% saccharin for 1 h in the morning, treated with nitroglycerin (50 mg/kg) or ethylene glycol dinitrate (EGDN) or corn-oil ($n = 10$) and later offered water for 1 hour. Groups differed little in intake of either fluid, and were left untreated on days 6 and 7. On day 8 they were offered saccharine given a novel flavour with coffee and dosed again. On day 9, corn-oil alone increased coffee intake by 6.1 ± 2.5 g, EGDN by 0.1 ± 1.5 g and nitroglycerin reduced it by 0.9 ± 2.1 g ($F_{2,27} = 3.39$, $P < 0.05$).

Effects in both social behaviour and conditioned aversion tests were in the predicted direction but were much smaller than expected, perhaps because the high solubility of nitroglycerin in corn-oil reduced the effective dose.

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The release of endogenous amino acids from the cat spinal cord *in vivo*

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Perfusion of the central canal of the cat spinal cord *in vivo* is a useful approach to the study of spinal neurotransmission (Morton, Stagg & Webster, 1976). Employing this technique, Jordan & Webster (1971) demonstrated the release of acetylcholine and radiolabelled glycine into the perfusate (in the presence of eserine and *p*-hydroxymercuribenzoate, respectively) during stimulation of femoral and sciatic nerves.

In the present experiments, cats were spinalized at C1 under halothane anaesthesia, immobilized with gallamine triethiodide and the central canal (L4–S1) was perfused (0.06 ml/min) with artificial CSF (Feldberg & Fleischhauer, 1960). A bipolar platinum electrode (tip separation 5 mm) was inserted into the cord at C2 to deliver trains (0.1 Hz, 5 s duration) of stimuli (12V, 2 ms, 40 Hz) for 10 min periods. Samples of perfusate were collected (10 min periods) and assayed for amino acids by a modification of the dansylation procedure described by Briel & Neuhoff (1972), utilizing [^{3}H]-dansyl chloride with [^{14}C]-leucine as an internal standard.

Table 1 shows those amino acids studied. Introduction of *p*-chloromercuriphenylsulphonate (pCMS) 10^{-4} M into the artificial CSF, in an attempt to inhibit amino acid uptake (Balcar & Johnston, 1973), significantly increased the efflux of several amino acids and further significant increases resulted from stimulation of descending spinal tracts. Stimulation in the absence of pCMS did not consistently increase the efflux of any amino acid studied (values not shown). The stimulated increases in glutamate, glycine, GABA and alanine efflux could be related to their proposed neurotransmitter roles (Curtis & Johnston, 1974) although the evoked release