Behavioural changes induced in mice following termination of ethanol administration

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The difficulty of assessing the efficacy of drugs in the treatment of ethanol dependence has been hindered by the lack of a suitable model. Experiments are described in which male T/O mice weighing 23–25 g receive ethanol orally or in vapour form. In the oral dosing experiment mice are on each of four successive days given intragastric injections of increased doses of ethanol (4, 5, 6 and 7 g/kg). Ethanol is administered as 40% w/v diluted with distilled water from 96% v/v.

The method used to expose the mice to ethanol vapour is similar to that described by Goldstein & Pal (1971) except that no alcohol dehydrogenase inhibitor (pyrazole) is given. Air is drawn through Perspex boxes at 2 l./minute. Ethanol (96% v/v) is metered into the air flow to give a vapour concentration of 20 mg/litre. This flow is continued for 8–14 days.

At the end of each treatment mice are grouped in fives and coloured to facilitate counting. Individual mouse head-twitch scores are recorded for periods of four minutes. Characteristic ethanol-withdrawal head-twitches are observed which reach a peak intensity about 12 h after blood ethanol concentrations fall to zero. Head-twitches were described by Corne & Pickering (1967) to occur in mice following the acute administration of hallucinogens.

'Grimaces' described by Goldstein could be induced, about 6 h after termination of ethanol vapour, by handling the mice but in our experiments there is a high incidence of this type of behaviour in control mice.

Analysis for significant differences between control and ethanol-treated animals is carried out on a quantal basis or using individual scores and the Mann-Whitney 'U' test.

REFERENCES

CORNE, S. J. & PICKERING, R. W. (1967). A possible correlation between drug-induced hallucinations in man and a behavioural response in mice. *Psychopharmacologia (Berl.)*, **11**, 65–78.

GOLDSTEIN, D. B. & PAL, N. (1971). Alcohol dependence produced in mice by inhalation of ethanol; grading the withdrawal signs. *Science*, **172**, 288–290.

An *in vivo* method for studying release of putative neurotransmitters from the rabbit olfactory bulbs

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A modified cortical cup technique (Mitchell, 1963) has been used to study release of the putative neurotransmitters noradrenaline (NA) and γ-aminobutyrate (GABA) (Muckart, 1971).

Essentially the method involves incubating the bulbs with Krebs solution containing either radioactive (±)-NA (7-^{3}H or methylene-^{14}C) and marker (^{14}C-urea or ^{3}H-inulin), or radioactive GABA (2,3-^{3}H) and ^{14}C-urea, or radioactive GABA and radioactive NA, together with appropriate additives to reduce NA and GABA breakdown (ascorbic acid 20 mg/l., EDTA 10 mg/l., amino-oxyacetic acid 10^{-5}M). Following this incubation, the efflux of radioactivity is measured by liquid scintillation counting. Labelled NA and its metabolites were separated by ion-exchange chromatography in some experiments. In other experiments the total counts attributable to the original labels were measured i.e. 'NA' and 'GABA'.

Two methods of stimulation were used, namely gross stimulation of the medial olfactory tract (MOT) using a bipolar silver balled electrode (0·5 ms pulses, 6 mA and 25–50 Hz), and more specific stimulation of the lateral olfactory tract (LOT) using a concentric bipolar electrode placed stereotaxically (0·2 ms pulses, 0·3 mA and 20–50 Hz). Using these parameters, stimulation for periods of 7 min (10 min collection periods) caused increases in the efflux of 'NA' (50%) and 'GABA' (40%) greater than in the preceding period. The ratio of labelled NA to labelled metabolites at rest (65–70% to 35–30%) was not significantly altered by stimulation. Metaraminol (10^{-4}M) had little effect on the resting efflux of 'NA', but significantly increased the size of the stimulation release (3 experiments, $P<0.01$ paired t-test).

Since the neurally evoked increase in the release of both 'NA' and 'GABA' was calcium-dependent and not accompanied by increase in release of the marker substances ^{3}H-inulin and ^{14}C-urea, the process appears to be specific.

Characteristically, the stimulated increases in release of both 'NA' and 'GABA' did not coincide with, but followed the period of stimulation. However, in experiments with labelled NA and lesions of the olfactory tracts caudal to the stimulating electrode, MOT stimulation induced release which coincided with the period of stimulation, and release was significantly larger than in non-isolated bulbs (5 experiments, $P<0.01$ paired t-test). These results suggest that normally some distant inhibitory influence may be responsible for delaying 'NA' release in response to tract stimulation.

In addition, the present experiments provide some evidence for a noradrenergic link in the neurally evoked release of GABA by olfactory tract stimulation. Thus the addition of cold NA (7×10^{-5}M) to the cup fluid in the presence of a high concentration of metaraminol (5×10^{-4}M) resulted in an increase in the spontaneous release of 'GABA', but not 'NA' or ^{14}C-urea. The increased release of 'GABA' which follows neural stimulation or addition of cold NA has not so far been consistently blocked by α-adrenoceptor antagonists (phentolamine 10^{-4}M; tolazoline 10^{-4}M). This is difficult to reconcile with iontophoretic studies which suggest that such antagonists should be effective (Salmoiraghi, Bloom & Costa, 1964).

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REFERENCES

MITCHELL, J. F. (1963). The spontaneous and evoked release of acetylcholine from the cerebral cortex. *J. Physiol., Lond.*, **165**, 98–116.

MUCKART, A. B. (1971). Neurally evoked release of noradrenaline from the olfactory bulb. *Br. J. Pharmac.*, **42**, 641–642P.

SALMOIRAGHI, G. C., BLOOM, F. E. & COSTA, E. (1964). Adrenergic mechanisms in rabbit olfactory bulb. *Am. J. Physiol.*, **207**, 1417–1424.

Chronic dorsal root section on free amino acid levels in the rabbit spinal cord

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Studies of the distribution of amino acids in the cat spinal cord (Graham, Shank, Werman & Aprison, 1967; Johnston, 1968) have implicated glutamate as the neurotransmitter at first sensory synapses, and aspartate and glycine as interneuronal transmitters. The effects of these amino acids applied iontophoretically are in accordance with these suggestions (Curtis & Watkins, 1960). In order to gain further information about the possible involvement of amino acids in spinal cord neurotransmission, we have studied the effect of chronic dorsal root section (DRS) on the gross levels of free amino acids in the spinal cord of the rabbit, since DRS should result in degeneration of primary afferent pathways and a reduction in associated transmitter substances.

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