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SHORT COMMUNICATIONS

The lack of effect on liver alcohol dehydrogenase in mice of early exposure to ethanol

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It has been shown that prenatal exposure of rats [1] and mice [2] to ethanol will change the behaviour of those animals during early adulthood even though exposure to ethanol ceased at birth. Sze *et al.* [3] found that alcohol dehydrogenase in the livers of adult mice of two strains (C57BL/10/Bg which is known to drink dilute solutions of ethanol in preference to water, and DBA/1/Bg which does not prefer ethanol solutions) was increased by 5 per cent in animals whose parents had received 10% ethanol in water from weaning up to 14 days after the birth of the litter in question. Such a long term effect by an exogenous substance on an enzyme is of some theoretical importance in the study of development, and the possible effects of maternal ethanol consumption on the human fetus have medical significance [4]. We thought it of importance to obtain confirmation of the results of Sze *et al.* [3] and to extend the results, if possible, to the alcohol dehydrogenase of brain which in rats is known to be very similar, if not identical, to the classical liver alcohol dehydrogenase [5, 6].

The strain of mouse used was C57BL/6J (originating at the MRC animal centre, Carshalton, U.K.). The animals were treated exactly as described by Sze *et al.* [3] for their EE and HH groups, i.e. EE parents received 10% ethanol in water as the only drinking fluid until 14 days after the birth of the litters in question, from which time they received only water. HH parents received only water to drink. The young were weaned at 24–25 days of age and were allowed access to water until they were killed at 48–50 days of age.

Alcohol dehydrogenase was assayed by two methods, fluorimetrically [3, 7] and colourimetrically as described by Raskin and Sokoloff [5]. In the fluorimetric assay the reduction of NAD^+ in the absence of any exogenous substrate was allowed to cease (10–15 min) before addition or ethanol. Protein was measured by the method of Lowry *et al.* [8] using bovine serum albumin dried over silica gel as the standard.

The colourimetric method of estimating alcohol dehydrogenase, which involves coupling the oxidation of ethanol to the reduction of lactaldehyde in the presence of NAD, followed by the development of a chromophore from the reaction product 1,2-propanediol, has distinct advantages over fluorimetric or direct spectrophotometric assays for this enzyme in crude centrifugal supernatants of tissue homogenates [9]. In the present case the predominant advantage is that the gross inaccuracies of measurement of NAD utilization in the presence of large quantities of endogenous substrates occuring in the enzyme preparation are completely avoided. In addition, it is not necessary to attempt to correct for the effect of aldehyde dehydrogenase in the cytosol preparation [7] if the colourimetric assay is used.

The C57BL/6J mice preferred 10% ethanol in water to water alone in a two bottle choice test made before the main experiments (water consumption = 30% of ethanol in water consumption).

Three separate experiments were done over a period of about 1 yr. In no case did the treatment described by Sze *et al.* [3] cause a significant increase in the activity of alcohol dehydrogenase in the livers from experimental animals when compared with that in controls. There was no significant difference in the body weights or in the liver weights of the animals in the two groups. The numerical values summarized by these statements of significance are grouped in Table 1. Alcohol dehydrogenase in brain is not affected by the ethanol exposure regime described above although during the period of ethanol administration to rats it is known to increase markedly [10].

The reasons for the discrepancy between our results and those of Sze *et al.* [3] are not clear. The strains of mouse used were very similar but not identical, and we used two assay systems based on different principles, one being identical to that of Sze *et al.* [3]. It is clear, however, that there is no well defined effect of ethanol on the activity of alcohol dehydrogenase in the livers of mice exposed to ethanol by the regime described above.

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REFERENCES

1. N. W. Bond and E. L. di Guisto, *Psychopharmacologia* **46**, 163 (1976).
2. J. Elis and M. Kršiak, *Activ. nerv. sup.* **17**, 281 (1975).
3. P. Y. Sze, J. Yania and B. E. Ginsburg, *Biochem. Pharmac.* **25**, 215 (1976).
4. Editorial, *Lancet* **i**, 1335 (1976).

Table 1. The activity of alcohol dehydrogenase in the cytosol of the livers from mice treated with ethanol prior to birth and during the first 14 days after birth

	Liver wt (g)	Body wt (g)	Enzyme activity (fluorimetric assay) nmole/min/mg of protein			Enzyme activity (coupled assay) nmole/min/mg of protein
Control	1.14 ± 0.05 [10]	17.96 ± 1.1 [11]	25.2 ± 0.48 [6]	27.9 ± 3.5 [5]	24.6 ± 4.2 [10]*	279 ± 34 [10]*
Ethanol treated	1.03 ± 0.13 [9]	16.91 ± 1.2 [10]	24.6 ± 0.42 [6]	25.4 ± 4.9 [5]	25.2 ± 2.2 [9]†	266 ± 36 [9]†

The values are means ± S.D. (number of individual mouse liver cytosol preparations). *,† represent values from the two assays performed on the same enzyme preparations. The coupled assay values are apparently 10-fold higher than the fluorimetric values because the coupling system circumvents the normal rate-determining cofactor dissociation [9].

5. N. H. Raskin and L. Sokoloff, *J. Neurochem.* **17**, 1677 (1970).
6. R. J. S. Duncan, J. E. Kline and L. Sokoloff, *Biochem. J.* **153**, 561 (1976).
7. S. Ohno, C. Stennius, L. Christian, C. Harris and C. Ivey, *Biochem. Genet.* **4**, 565 (1970).
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
9. R. J. S. Duncan, *J. Neurochem.* **28**, 681 (1977).
10. N. H. Raskin and L. Sokoloff, *Nature, New Biol.* **236**, 138 (1972).

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Microinjection of opioids into the nucleus reticularis gigantocellularis of the rat: Analgesia and increase in the normetanephrine level in the spinal cord

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In studies in which intracerebral microinjections of morphine were given, it was noted that several regions of the brain stem, e.g. the hypothalamus[1, 2], the periaqueductal gray matter[3–5], the floor of the fourth ventricle[6] and the ventral surface of the brain stem[7], are all involved in the antinociceptive action of this narcotic.

Recently, Takagi *et al.*[8, 9] found that, when they gave microinjections of morphine into the nucleus reticularis gigantocellularis (NRGC) of the medulla oblongata of rats, analgesia was produced in a dose-dependent manner. The ED_{50} value was 0.038 μg, and with even the small dose of 0.015 μg, a definite analgesia resulted[9]. These doses are considerably less than those (2–200 μg) used in other studies[1–7] in which intracerebral microinjections of morphine have been given.

In neurochemical studies carried out in our laboratory[10], it was noted that systemic injection of morphine increased the concentration of normetanephrine (NM), a metabolite of noradrenaline (NA), in the dorsal half (mainly the dorsal horn) of the rat whole spinal cord, but not in the ventral half (mainly the ventral horn). Moreover, it was observed that the NM increasing effect of morphine disappeared after transection of the spinal cord at C-1, but not after transection of the brain stem at the inter-collicular level. From these results it was concluded that the primary site of the NM increasing action of morphine is in the lower brain stem where noradrenergic cell bodies are located, and that morphine causes an increase in the neuronal impulse flow of the bulbospinal noradrenergic system which in turn enhances the release of NA, a possible inhibitory transmitter at the spinal dorsal horn [11, 12].

These observations prompted us to investigate the effect of microinjections of morphine into the NRGC of the medulla oblongata on the level of NM in the spinal cord of the rat.

Experiments were carried out on male Wistar rats (170–240 g). At least 1 week before testing, a guide cannula was unilaterally implanted into the cerebellum and positioned 4 mm above the intended site of injection as described previously [8]. Morphine HCl (0.5 μg in 0.5 μl of physiological saline) or methionine-enkephalin (10 μg in 0.5 μl of distilled water) was injected through the injection cannula which had been inserted into the guide cannula to protrude 4 mm beyond the end of it so that the tip was introduced into the NRGC. The injection cannula was withdrawn 10 sec after an injection period of 30 sec. The stereotaxic coordinates of the NRGC (AP, 10.0–10.5, L, 1.0, H, 9.7), including the nucleus reticularis paragigantocellularis, were determined according to the atlas of Fifková and Maršala [13]. The position of the injection site was verified histologically.

The antinociceptic action of morphine was evaluated by the tail-pinch method [8]. Hemostatic forceps (3 mm in width and 2 kg constant pressure) were applied at the base of the tail. Analgesic effects were scored as follows: 1, 0.5 and 0 scores were given if the latent periods of the biting response to the forceps were more than 10, 5–10 and less than 5 sec respectively.

The method of removal of the spinal cord was as described by Shiomi and Takagi [10]. The extraction and fluorimetric determinations of NM and NA in the whole spinal cord were carried out by the methods of Anton and Sayre [Refs. 14 and 15 respectively]. Statistical significance was determined by Student's t-test.

Although injection of the vehicle (0.5 μl of physiological saline) into the NRGC exerted no significant effect on the nociceptive response of the rat to tail-pinch, microinjection of morphine in a dose of 0.38 μg (0.5 μg as morphine HCl) produced a marked antinociceptive effect (Fig. 1b). The effect reached a maximum within 5 min and the nociceptive response to the tail-pinch recovered after 90 min. This is consistent with our previous reports[8, 9].

The measurement of NM was used as an index of the activity of noradrenergic neurons, since NM is considered to be formed by catechol - *O* - methyltransferase from NA after its neuronal release [16]. Microinjection of morphine in a dose of 0.38 μg into the NRGC increased the level of NM in the spinal cord (Fig. 1a) with no effect on that of NA. This indicates the increased activity of the noradrenergic system in the spinal cord. The increase in the level of NM in the spinal cord reached a peak 5 min after injection of morphine and disappeared after 30 min. This time course corresponds relatively with that of morphine analgesia (Fig. 1, panels a and b). Thus, the NM enhancing action and analgesia of morphine injected into the NRGC were more rapid in onset and shorter in duration than that injected systemically[10]. The NM levels after microinjection of morphine, in doses of 0, 0.076, 0.15 and 0.38 μg, into the NRGC were dose-dependent: 23.4 ± 2.1 ($n = 15$), 29.9 ± 4.4 ($n = 13$), 32.7 ± 3.7 ($n = 18$) and 37.7 ± 5.1 ng/g (mean ± S. E. M.) ($n = 14$) respectively. Injection of the vehicle (0.5 μl) into the NRGC had no significant effect on the NM level in the spinal cord (Fig. 1a). Pretreatment of rats with naloxone HCl (1 mg/kg, s.c.) 10 min before microinjection of morphine (0.38 μg) into the NRGC completely prevented the NM increase induced by morphine.

Endogenous opioid peptides, when injected into the NRGC of the rat, have a potent analgesic action[17]. We examined the effect of methionine-enkephalin, injected into the NRGC, on the level of NM in the spinal cord of the rat. As shown in Fig. 2, methionine-enkephalin (10 μg) significantly increased the level of NM 5 min after the microinjection. The vehicle (0.5 μl) injected into the NRGC produced no significant effect on the level of NM in the spinal cord. The NM-increasing effect of methionine-enkephalin was blocked by naloxone HCl (1 mg/kg, s.c.) administered 10 min before microinjection of the peptide. These results indicate that both morphine and methionine-enkephalin injected into the NRGC ele-