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SHORT COMMUNICATIONS

Monoamine oxidase in germ-free chicks: increased activity in liver but not brain

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In 1962 Phillips *et al.* [1] noted a significantly higher activity of monoamine oxidase (EC 1.4.3.4; MAO) in the liver of germ-free chicks compared with conventionally bred controls. In this study we have tried to confirm and extend their work, measuring enzyme activity in liver and brain with 5-hydroxytryptamine (5-HT) and phenylethylamine (PEA) as substrates specific, respectively, for the A and B forms of the enzyme in most (but not all) tissues [2]. The activity of succinate dehydrogenase (SDH), another enzyme located like MAO in the mitochondrial outer membrane, was also measured.

Chicks of Rhode Island Red × Light Sussex cross were reared in the two environments to 14 days of age. They received a practical-type diet based on soya and maize meals, sterilised by gamma-radiation. They were killed by fracture of the neck, and brain and liver were quickly removed, weighed and dropped into liquid nitrogen. These organs were homogenized respectively in 10 and 20 vol 0.1 M sodium phosphate buffer (pH 7.4). MAO activity was measured as described previously [3] using [2-^{14}C]5-HT (0.34 mM) and [2-^{14}C]PEA (0.028 mM) as substrate and blanks were prepared by adding the selective MAO A or B inhibitors, clorgyline and deprenyl (10^{-3} final concentration), to buffer and homogenates before incubation. Succinate dehydrogenase (SDH) was estimated by Pennington's method [4] scaled down by half. Labelled 5-HT (50 mCi/mmol) was obtained from the Radiochemical Centre, Amersham, U.K. and PEA (51 mCi/mmol) from New England Nuclear Corp., Winchester, U.K. Other chemicals were purchased from Sigma, London. Protein was estimated according to Lowry *et al.* [5] using bovine serum albumin as standard. All assays were carried out in duplicate.

Means values for body weight, liver and brain weight and protein content did not differ between germ-free and control chicks. However, MAO activity was significantly raised in the liver of germ-free chicks. Both 5-HT and PEA-oxidizing ability were increased to a similar extent, 47 per cent and 39 per cent respectively (Table 1), so that there was no selective enhancement in activity. SDH activity, on the other hand, was reduced in germ-free chicks, although not significantly so. Unlike liver, there was no significant difference between brains of germ-free and control chicks for any of the parameters measured (Table 2).

We thus confirmed the findings of Phillips *et al.* [1] that liver MAO activity is increased in germ-free chicks. As SDH activity is, if anything, lower in these birds, the finding suggests that the increase is specific to MAO rather than a reflection of change in all mitochondrial enzymes. The difference is presumably a manifestation either of greater enzyme induction in germ-free animals or of an inhibitor in the conventional chicks. If the former, there is little pertinent information available pointing to a possible mechanism; although there is evidence that a pharmacologically active substance or substances are elaborated in the caecum of germ-free rodents [6], it is not known whether the same is true for the chick. If the latter, then we have to fall back on an interpretation in terms of an inhibitor being produced by gut flora from whence it passes directly to the liver. Presumably, this inhibitor, if it exists, does not pass into the brain, despite the relative absence of blood–brain barrier in immature animals of this species [7]. If such an inhibitor did exist, it might be rewarding to search for it in other peripheral organs, particularly the blood platelet, which has been widely used as an index of *in vivo* MAO inhibition [8]. It is of particular interest in this connection that Berrettini and Vogel [9] have recently described what appears to be an endogenous MAO inhibitor in schizophrenic subjects responsible for the deficit they observed in platelet activity. In view of a somewhat tenuous but persistent literature drawing attention to the possible importance of gastrointestinal malabsorption in the patho-

Table 1. Monoamine oxidase and succinate dehydrogenase activity (nmol/mg protein/30 min ± S.D.) in liver

	No. of chicks	Mean of body weight (g)	Mean of liver-weight (g)	5-HT-oxidizing activity	PEA-oxidizing activity	SDH
Control	8	127	3.82	12.07 ± 5.3	3.37 ± 1.0	0.9 ± 0.23
Germ-free	8	132	3.76	22.86 ± 5.0	5.48 ± 0.9	0.73 ± 0.15
				$P < 0.0025$	$P < 0.0005$	$P < 0.10$

Table 2. Monoamine oxidase and succinate dehydrogenase activity (nmol/mg protein/30 min ± S.D.) in brain

	No. of chicks	Mean of body weight (g)	Mean of brain weight (g)	5-HT-oxidizing activity	PEA-oxidizing activity	SDH
Control	8	127	1.42	25.7 ± 5.5	11.6 ± 3.4	0.21 ± 0.01
Germ-free	8	132	1.46	26.7 ± 4.9	13.3 ± 0.2	0.19 ± 0.03

genesis of schizophrenia [10, 11], it may just be worth while turning our attention to the gut flora in this context.

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Changes induced by DMet^2-pro^5-enkephalinamide in plasma insulin response to glucose in the rat

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From reports of several laboratories, it seems reasonable to assume that opioid peptides, endogenous ligands for opiate receptors, may operate as well as in pain modulation, in regulating extrapyramidal, limbic system and neuroendocrine functions (for a review see [1]).

Previous work from this laboratory [2, 3] has shown that leu-enkephalin stimulates a release of growth hormone as well as of prolactin from pituitary, and this agrees with results obtained with Met-enkephalin by Rivier *et al.* [4].

We now report an extrapituitary hormonal effect of an opioid. D-Met^2-pro^5-enkephalinamide, an analogue of met-enkephalin with long lasting analgesic activity [5], causes changes in insulin levels in plasma of glucose-loaded rats.

Male Sprague–Dawley rats weighing 220 ± 20 g were used, and housed under standard conditions (room temperature 20°, relative humidity 60 per cent). Studies were begun between 8 and 9 a.m. following an overnight fast. Administration of the drug and withdrawal of blood were accomplished in unanesthetized and unrestrained rats. This was made possible by using animals with permanent indwelling jugular cannula, according to the method of Cocchi *et al.* [6]. A group of ten rats (controls) received intravenously 1 ml/kg of a 33 per cent glucose solution in saline. Another group of ten animals received intravenously, immediately after an identical load of glucose, 0.4 mg/kg of D-Met^2-pro^5-enkephalinamide (Met-Enk-NH_2) dissolved in saline.

Blood samples were taken from each group of animals at various time-intervals, for the determination of plasma insulin by radioimmunoassay, according to the method of Keane *et al.* [7]. Rat insulin was used as a standard (Novo Industri, DK). From the same blood samples, glucose concentration was also determined, according to the method of Werner *et al.* [8].

The results reported in Table 1 indicate that a rise occurs, as usual, in plasma insulin levels of glucose-loaded rats, with a peak effect under our experimental conditions, at the 3rd min.

Table 1. Plasma insulin levels (IRI) of glucose loaded rats following the administration of a single dose of D-met^2-pro^5-enkephalinamide (Met-Enk-NH_2, 0.4 mg/kg/i.v.)

Time of observation (min)	IRI (μUnits per ml)	
	Glucose load (controls)	Glucose load + Met-Enk-NH_2
0	28.2 ± 1.7	26.4 ± 2.3
3	127.5 ± 8.3	76.3 ± 3.7*
8	52.3 ± 2.4	71.4 ± 2.9*
15	34.9 ± 1.9	29.8 ± 1.4
30	21.2 ± 1.1	26.3 ± 1.8
60	27.4 ± 1.7	32.8 ± 1.5

* $P < 0.01$ compared with control values at the same time-intervals. The values are the means of 10 animals ± S.E.M.

Table 2. Glycemic values of glucose loaded rats treated or not with D-met^2-pro^5-enkephalinamide (Met-Enk-NH_2, 0.4 mg/kg/i.v.)

Time of observation (min)	Glycemia (mg%)	
	Glucose load (controls)	Glucose + Met-Enk-NH_2
0	96.5 ± 5.2	88.3 ± 3.4
3	247.4 ± 12.6	322.9 ± 11.7*
8	143.2 ± 10.9	197.8 ± 9.5*
15	91.5 ± 5.3	164.4 ± 8.7*
30	102.7 ± 4.8	116.8 ± 5.9
60	108.3 ± 4.5	91.3 ± 4.1

* $P < 0.05$ compared with control values at the same time-intervals. The values are the means of 10 animals ± S.E.M.