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## Inhibition of median raphe neurone metabolism by cerebrospinal fluid (CSF) containing 5-hydroxytryptamine and melatonin

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BRAIN 5-hydroxytryptamine is synthesized by many neurones of the raphe nucleus in the midbrain. For several hours after monoamine oxidase inhibition the rate of 5-hydroxytryptamine synthesis declines as the level of brain 5-hydroxytryptamine rises.[1,2] In the present study the nucleolar component of the metabolic changes in median raphe neurones was observed (a) during a prolonged (9 day) elevation of 5-hydroxytryptamine levels in cerebrospinal fluid (CSF), (b) during a similar elevation in CSF levels of other monoamines, (c) after depletion of brain catecholamines by 6-hydroxydopamine (6-OHDA),[3] and (d) after bilateral cervical sympathectomy.

The cerebral ventricles of one group of male albino rats (aged 3 months and weighing approximately 300 g) were cannulated and continuously infused during 7–9 days, at a rate (1 $\mu$l/min) roughly half that of CSF formation.[4] The infusate was artificial CSF alone, or with "low-dose" 5-hydroxytryptamine, melatonin, noradrenaline, histamine (all at 50 $\mu$g/ml), tryptophan (1 mg/ml), or "high-dose" 5-hydroxytryptamine (500 $\mu$g/ml). The rats were killed after 7–9 days of infusion.

In a second group of rats, 6-OHDA (250 $\mu$g in 0·05 ml artificial CSF) was given by a single injection into a lateral cerebral ventricle; controls received artificial CSF alone. The animals were killed at intervals during the following 16 days.

In a third group, bilateral cervical sympathectomy was performed and the rats killed 7–9 days thereafter.

From all rats, neuronal nuclei were isolated from the median raphe region of the brain.[5] The dry mass of the nucleoli in these nuclei was measured using the interference microscope,[6] and the mean values are shown in Fig. 1 and Table 1.

Although the drug-induced behaviour of the rats within each group was similar, differences between the groups were seen. Two days after commencing infusion with melatonin or with "low-dose" 5-hydroxytryptamine the rats became very drowsy and showed a reduction both in spontaneous motor activity and in reactivity to simple tactile and auditory stimuli. During infusion with "high-dose" serotonin the rats were drowsy in the first day only, subsequently becoming hyperactive and hyperreactive with a coarse tremor and titubation. Rats with noradrenaline or tryptophan in the infusate showed increased spontaneous movements and hyperreactivity starting on day 2 but not preceded by drowsiness. For 1 hr after 6-OHDA injection the rats were hyperactive and hyperreactive; the subsequent 12-hr phase of extreme immobility and unresponsiveness disappeared gradually, behavioural normality returning during day 2. Those rats with cervical sympathectomy and those receiving histamine solution or artificial CSF by itself appeared normal. Assiduous attempts have been made by many workers to attribute such drug-induced changes in behaviour to concurrent changes in brain biochemistry.[7,8] It is hoped that such speculations might be assisted by this study in which accompanying changes in neuronal metabolism have been noted by means of a different technique.

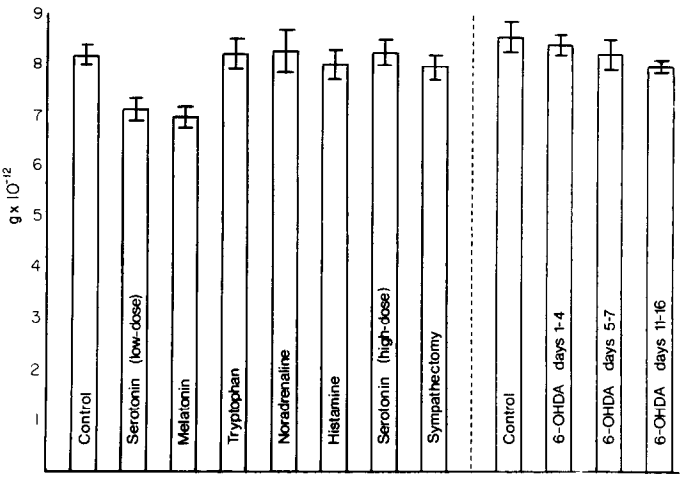


FIG. 1. Raphe neurones: columns indicate pooled mean nucleolar dry mass measurements with their standard errors, grouped according to experimental procedure.

A cell's metabolic capacity for synthesizing protein depends largely on its complement of ribosomal RNA. The varying rate of ribosomal RNA synthesis necessary in changing circumstances can be related quantitatively to alterations in the cell's nucleolar dry mass.[6,9,10] Thus from the measurements of the nucleolar dry mass of raphe neurones in this study (Fig. 1, Table 1) the following deductions can be drawn: (1) raphe neurone metabolism is depressed by the prolonged infusion of 5-hydroxytryptamine ("low-dose") and of melatonin, two hormones which are biochemically closely related; (2) the raphe neuronal inhibition produced during melatonin infusion is unlikely to be secondary to changes in pineal metabolism; for although the amount of melatonin synthesized by the pineal gland is altered by bilateral cervical sypathectomy,[11] in this study the same procedure does not alter the raphe parameter; (3) the inhibition induced during 5-hydroxytryptamine and melatonin infusion is not mediated by changes in the metabolism of neurones synthesizing other monoamines; this is indicated by the stability of the raphe parameter during the noradrenaline, 6-OHDA and histamine experiments; (4) the similar stability in the raphe parameter during tryptophan infusion demonstrates that an increased availability of precursor does not alter the nucleolar metabolism of raphe neurones; (5) "high-dose" 5-hydroxytryptamine infusion abolishes the inverse relationship noted here and elsewhere[1,2] between the rate of raphe neurone metabolism and brain levels of 5-hydroxytryptamine.

TABLE 1.

| Procedure | Nucleolar dry mass ($g \times 10^{-12}$) | No. of observations and animals | P values |
|---|---|---|---|
| Infusion control | 8·17 ± 0·29 | 205; 4 | — |
| 5-Hydroxytryptamine (low dose) | 7·12 ± 0·24 | 333; 6 | 0·01–0·005 |
| Melatonin | 6·98 ± 0·20 | 358; 5 | <0·001 |
| Tryptophan | 8·23 ± 0·33 | 257; 5 | N.S. |
| Noradrenaline | 8·28 ± 0·45 | 104; 2 | N.S. |
| Histamine | 8·02 ± 0·29 | 237; 3 | N.S. |
| 5-Hydroxytryptamine (high dose) | 8·24 ± 0·25 | 328; 4 | N.S. |
| Sympathectomy | 7·96 ± 0·25 | 262; 3 | N.S. |
| 6-OHDA Control | 8·55 ± 0·30 | 198; 3 | — |
| 6-OHDA d1–4 | 8·40 ± 0·22 | 268; 4 | N.S. |
| d5–7 | 8·22 ± 0·28 | 187; 3 | N.S. |
| d11–16 | 7·95 ± 0·16 | 535; 7 | (0·1–0·05) N.S. |

N.S.—not significant.

It is not certain what physiological mechanisms decrease the tempo of 5-hydroxytryptamine production in raphe neurones when the level of brain 5-hydroxytryptamine rises. The findings in this study support the hypothesis[12,13] that receptors relatively specific for 5-hydroxytryptamine are situated distal to raphe nerve endings; these receptors' neurones control, by feedback inhibition, an excessive rate of 5-hydroxytryptamine production by the raphe neurones as evidenced by moderately raised levels of 5-hydroxytryptamine at the receptors. This study further suggests that, at very high levels of 5-hydroxytryptamine, these receptors become saturated and inactivated; in this situation the inhibitory feedback control is eliminated. Experiments now in progress are designed to test the validity of this hypothesis.

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## Species differences in lipid peroxidation and their effects on ethylmorphine *N*-demethylase activity in liver microsomes

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WE HAVE recently demonstrated the close inverse relationship between lipid peroxidation and activities of drug-metabolizing enzymes in liver microsomes of rats.[1] Regarding the changes in ethylmorphine *N*-demethylating activity, the activity was decreased when microsomes were incubated in the presence of ferrous ion, and was apparently increased by incubation of microsomes with inhibitors of lipid peroxidation such as EDTA, *o*-phenanthroline, $\alpha,\alpha'$-dipyridyl and $Co^{2+}$. The present study was initiated in order to examine further the effects of EDTA and ferrous ion on lipid peroxidation and activity of ethylmorphine *N*-demethylase in liver microsomes from various species.

Thiobarbituric acid (TBA) and ethylmorphine were purchased from commercial sources and used without further purification. Thiobarbituric acid solution (0·67%) was prepared as previously described.[1] NADP, glucose 6-phosphate-Na and glucose 6-phosphate dehydrogenase (EC 1.1.1.49, gradc I, Kontroll-Nr. 7291111) were purchased from Boehringer Mannheim (Japan) Co. Ltd.

Male rats (Sprague–Dawley strain) weighing 70–90 g, male mice of dd strain weighing 23–27·5 g, male guinea pigs weighing 320–340 g, and male albino rabbits weighing 2·25–2·61 kg were maintained on commercial chow, and were starved for about 18 hr prior to sacrifice. Three livers of rats and 12 livers of mice were pooled and used for each respective experiment. Liver microsomes of the animals were prepared by a method previously described.[1]