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SHORT COMMUNICATIONS

The effect of disulfiram, diethyldithiocarbamate and dimethyldithiocarbamate on serotonin and 5-hydroxyindole-3-acetic acid brain levels in rats

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DISULFIRAM (DS), diethyldithiocarbamate (EE) and dimethyldithiocarbamate (MM) inhibit the activity of dopamine-β-hydroxylase[1, 2] and lower the noradrenaline level in various tissues including the brain.[3–6] Consequently they are used to investigate the role of catecholamines (CA) in the action of various drugs. The specificity of their action has, however, been questioned.[7–9]

While investigating the activity of dopamine-β-hydroxylase inhibitors it was decided to attempt to discover whether this group influences the serotonin (5-HT) as well as the CA level in brain. The literature dealing with this problem is both scanty and contradictory. It was reported that EE did not alter the 5-HT level in rat intestine,[10] and that DS did not influence the activity of tryptophan hydroxylase in rat brain.[11] However, it was also found that DS lowered the 5-hydroxyindole acetic acid (5-HIAA) level in rat liver *in vitro*[12] and that EE at doses higher than those used by us increased the 5-HT level in rabbit brain[13] but not in intestine.[10] The brain content of 5-HT in rats was determined only by Spencer and Turner,[14] who found that EE (400 mg/kg) had no effect.

Methods and materials

The experiments were carried out on albino Wistar rats. DS (suspension in 3% Tween 80) was administered intraperitoneally three times in 2-hr intervals, EE and MM (in saline) were given in single doses. Rats were killed by transcervical dislocation 3 hr after the last injection of DS or 2 hr after EE or MM. Our previous studies indicated that at those times the depression of NA was at its greatest.[5] Groups of rats used for determination of 5-HT and 5-HIAA in whole brain consisted of six to eight subjects; groups used for determination of 5-HT in individual brain regions (dissected according to Popov *et al.*[15]) consisted of twenty animals. The regions from four brains were pooled for assay. 5-HT was determined with the method of Ansell and Beeson,[16] and 5-HIAA in the second half of the brain, with the method of Udenfriend *et al.*[17]

Statistical evaluation was performed using Student's *t*-test.

TABLE 1. EFFECT OF DISULFIRAM (DS), DIETHYLDITHIOCARBAMATE (EE) AND DIMETHYLDITHIOCARBAMATE (MM) ON THE BRAIN 5-HT AND 5-HIAA IN THE RAT

Treatment (mg/kg)	5-HT (μg/g) $\pm$ S.E.	5-HIAA (μg/g) $\pm$ S.E.
control*	0·473 $\pm$ 0·05	0·489 $\pm$ 0·09
DS 3 $\times$ 50	0·504 $\pm$ 0·08	0·585 $\pm$ 0·11
DS 3 $\times$ 100	0·332 $\pm$ 0·04	0·597 $\pm$ 0·11
control†	0·514 $\pm$ 0·06	0·418 $\pm$ 0·06
EE 250	0·491 $\pm$ 0·07	0·599 $\pm$ 0·04‡
EE 500	0·386 $\pm$ 0·03‡	0·480 $\pm$ 0·04
MM 250	0·476 $\pm$ 0·07	0·532 $\pm$ 0·07
MM 500	0·506 $\pm$ 0·04	0·614 $\pm$ 0·11

The data are means of six to eight determinations made 3 hr after the last injection of DS or 2 hr after EE or MM.
* 3% Tween 80 i.p.
† Saline i.p.
‡ Different from the control: $P < 0·05$.

Results

The results of determinations in whole brain are presented in Table 1. In most cases the investigated compounds did not significantly alter the levels of 5-HT and 5-HIAA. Only in two groups were significant changes noted: EE 250 mg/kg caused an increase in 5-HIAA content (to 143 per cent) without influencing the 5-HT content, and EE 500 mg/kg lowered the 5-HT content (to *ca.* 75 per cent of control level) without affecting the 5-HIAA level.

TABLE 2. EFFECT OF DISULFIRAM (DS) DIETHYLDITHIOCARBAMATE (EE) AND DIMETHYLDITHIOCARBAMATE (MM) ON THE 5-HT CONTENT IN DIFFERENT REGIONS OF THE RAT BRAIN

Brain region	5-HT content (μg/g $\pm$ S.E.)			
	control	DS 3 × 100 (mg/kg)	EE 500 (mg/kg)	MM 500 (mg/kg)
cortex	519·7 ± 30·1	533·3 ± 58·8	483·9 ± 29·9	514·3 ± 35·0
striatum	424·2 ± 10·5	432·5 ± 85·6	404·3 ± 12·4	461·2 ± 23·2
Thalamus	1076·2 ± 58·9	801·5 ± 55·6†	947·3 ± 51·8	779·1 ± 71·5*
hypothalamus	1341·9 ± 80·9	1641·2 ± 117·3	1594·9 ± 86·2	1699·7 ± 71·8*
hippocampus	764·0 ± 42·8	733·8 ± 47·6	567·3 ± 34·5†	690·6 ± 68·8
colliculi + tegmentum	1502·7 ± 36·2	1474·8 ± 20·9	1494·1 ± 32·6	1588·0 ± 19·6
pons + medulla oblongata	1453·3 ± 258·1	1683·9 ± 328·4	1480·2 ± 155·8	1735·5 ± 134·7

The data are means of five determinations. Each determination was made in pools from four animals. The rats were killed 3 hr after last injection of DS or 2 hr after EE or MM.
Different from the control: *$P < 0.05$, †$P < 0.01$.

Significant effects of DS, MM and EE on 5-HT levels in various brain structures were noted only in a few cases (Table 2). DS and MM lowered the 5-HT level in thalamus (to 74 and 72 per cent of the control level resp.). EE lowered the 5-HT content of hippocampus (to 74 per cent) and MM enhanced the 5-HT level in hypothalamus to 72 per cent. As these effects are rather small and not unidirectional, and the employed doses were large it might be assumed that the investigated inhibitors do not significantly influence the 5-HT level in the investigated structures.

The results of the investigations in the whole brain and its structures seem to indicate that DS, EE and MM do not affect the 5-HT level in rat brain.

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Effects of papaverine derivatives on cyclic AMP phosphodiesterase of human platelets

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AGGREGATION of human platelets is inhibited by adenosine-3′,5′-monophosphate (cyclic AMP) and by its dibutyryl derivative.[1] These results suggest that cyclic AMP may be important in the regulation of platelet adhesiveness. Accordingly, the levels of intracellular cyclic AMP may play a major role in the susecptibility of platelets to aggregation and, ultimately, in thrombogenesis. The level of cyclic AMP is determined both by the activity of adenyl cyclase catalyzing the conversion of ATP to cyclic AMP, and by the activity of a specific phosphodiesterase (PDE) hydrolyzing cyclic AMP to adenosine-5′-monophosphate. Certain agents known to affect platelet aggregation are known to alter adenyl cyclase or phosphodiesterase activity in platelets. It was shown that stimulation of β-receptors by isoprenaline causes platelet clumps to disaggregate and that such a response is mediated by increased formation of cyclic AMP.[2] Prostaglandin E_1 inhibits platelet aggregation and stimulates cyclic AMP synthesis by human platelet membrane fractions apparently by stimulation of adenyl cyclase.[3, 4] On the other hand, theophylline inhibits platelet PDE resulting in increased cyclic AMP levels.[2] This drug is synergistic with isoprenaline in the inhibition of platelet aggregation.[2] We found that papaverine and some of its derivatives impair platelet functions most important for hemostasis and thrombosis.[5, 6] Platelets treated with papaverine loose their ability to adhere and to aggregate. Furthermore, phosphodiesterase activity in other tissues was found to be inhibited by papaverine.[7] Therefore, we investigated the possibility whether the inhibition of platelet aggregation by papaverine was mediated by an inhibition of platelet PDE and the subsequent increase of cyclic AMP.

The procedure used was that described by Butcher and Sutherland.[8] It involves conversion of 3′-5′ cyclic AMP to 5′ AMP, hydrolysis of the latter with excess of 5′-nucleotidase and measuring the liberated inorganic phosphate.[9] A whole platelet lysate suspension obtained by freezing and thawing of cells previously washed in saline two times and resuspended in a mixture of 2×10^{-3} M glycylglycin buffer, pH 7·4, 1×10^{-3} M $MgSO_4$, 0·01 M NaCl, and 0·01 M KCl was used for enzyme assay.

It was found that low concentrations of papaverine inhibit the PDE activity of platelet lysates. As shown in the dose–response curve (Fig. 1), 0·044 μM of papaverine/ml produce a 50 per cent inhibition of 3′-5′ cyclic AMP hydrolysis. In other experiments the effect of papaverine derivatives described as effective inhibitors of platelet aggregation[5] was compared with theophylline, a known inhibitor of 3′-5′ cyclic AMP PDE. The results are summarized in Table 1. It has been shown that all derivatives investigated are potent PDE blockers which surpass the effectiveness of theophylline.

The results support the assumption that aggregation is inhibited by substances able to induce 3′-5′ cyclic AMP accumulation in platelets.