Nature (Lond.) 230, 532-533 (1971).

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observation indicates that a unique population of human lymphocytes is involved, and that perhaps the same receptor binds both kinds of red cells.

This observation is an example of an interaction between a lymphoid cell and a heterologous erythrocyte which, although having some degree of specificity, is probably not immunological in nature for several reasons. First, a very high proportion of peripheral blood lymphocytes bind SRBC. It would certainly be difficult to admit that 20–40% of the lymphocytes have anti-SRBC antibodies on their membranes. Second, many of the donors had "natural" antibodies in the serum directed against SRBC or PRBC, but no correlation could be established between the titres of antibodies and the percentage of rosettes found among their lymphocytes. A patient with mononucleosis was tested while her titre of anti-SRBC in the serum was still high (1 : 112) but the percentage of rosettes was in the normal range (24.6%). Third, although the same population of human lymphocytes bound both PRBC and SRBC, serum antibodies against these kinds of erythrocytes did not cross react, as could be determined by absorption experiments. Fourth, this phenomenon has peculiar temperature requirements, which are different from those reported for cluster formation between erythrocytes and lymphoid cells from immunized animals[3].

Another implication of these findings is that SRBC or PRBC may interact with human lymphoid cells when they are cultivated together *in vitro*. Actually a low grade incorporation of isotopically labelled thymidine has been reported in such cultures[4,5], which was abolished if the erythrocytes were disintegrated by ultrasound. The observation described here may also be relevant to the findings of Bach *et al.*[6,7], who noticed that a few human peripheral blood lymphocytes (0.4–2.6%) form rosettes when incubated with SRBC. They found that this interaction is inhibited by anti-lymphocytic sera and suggested that the immunosuppressive potency of these sera could be measured by their rosette inhibitory titres. The experimental conditions used by Bach *et al.* differ from those here described, and so the relationship between their findings and ours remains to be clarified. Finally, because only part of the circulating lymphocytes are involved in cluster formation, it would be important to establish whether they constitute a separate sub-population of cells, as suggested by the larger proportion of clusters among thymocytes and their absence among some cultivated Burkitt lymphoma cells.

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Note added in proof. Two papers have recently been published describing the same phenomenon: Brain, P., *et al.*, *Clin. Exp. Immunol.* **6**, 681 (1970); Coombs, R. R. A., *et al.*, *Int. Arch. Allergy*, **39**, 658 (1970).

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1 Bianco, C., Patrick, R., and Nussenzweig, V., *J. Exp. Med.*, 132, 702 (1970).
2 Brittinger, G., Hirshhorn, R., Douglas, S. D., and Weissmann, G., *J. Cell Biol.*, 37, 394 (1968).
3 Biozzi, G., Stiffel, C., and Mouton, D., in *Immunity, Cancer and Chemotherapy* (edit. by Mihich, E.) (Academic Press, New York, 1967).
4 Hardy, D. A., and Lyng, N. R., *Nature*, 221, 545 (1969).
5 Möller, G., *Clin. Exp. Immunol.*, 4, 65 (1969).
6 Bach, J. F., Dormont, J., Dardenne, M., and Balner, H., *Transplantation*, 8, 265 (1969).
7 Bach, J. F., *Fed. Proc.*, 29, 120 (1970).

Effect of N,N-Dimethyltryptamine and D-Lysergic Acid Diethylamide on the Release of 5-Hydroxyindoles in Rat Forebrain

Kopien für

SEVERAL investigators have already shown that electrical stimulation of the mid-brain raphé, in which neuronal perikarya containing 5-hydroxytryptamine (serotonin) are almost exclusively located[1], produces a significant increase in the forebrain content of 5-hydroxyindolyl-3-acetic acid (5-HIAA), while the concentration of serotonin decreased or remained unchanged[2–6]. This communication describes how N,N-dimethyltryptamine (DMT) and D-lysergic acid diethylamide (LSD), the two potent psychotogenic drugs, affect the release of serotonin and 5-HIAA in the forebrain when the mid-brain is stimulated. Both drugs uniformly inhibit the response of single units in the mid-brain raphé nuclei[7,8].

Adult rats of both sexes (180–250 g) were anaesthetized lightly with intraperitoneal urethane (1.0 g/kg of weight), and a bipolar steel stimulating electrode was placed stereotaxically in the caudal mid-brain raphé[9] in stimulated and unstimulated rats at A (anterior) 0.4 mm; L (lateral) 0.0 mm and H (horizontal)—2.6 mm. The correct placement of each electrode was confirmed histologically. Stimuli were monophasic square wave pulses (10 Hz, 2 ms in duration, 4–6 V) applied for 40 min from a Grass S8 stimulator. N,N-dimethyltryptamine (Sigma) was administered intraperitoneally (36 mg/kg) at the start of stimulation. LSD (0.6 mg/kg, Sandoz) was given intraperitoneally in two equal doses; the first at the onset of stimulation and the second 20 min after the start of stimulation. This mode of administration was chosen because the effect of LSD on the metabolism of serotonin correlates with the time course of clearance of the drug from the brain. At corresponding times, control animals received equal volumes of saline. After the cessation of stimulation, rats were decapitated and the forebrains were removed immediately and frozen at −20° C. The serotonin and 5-HIAA content of the forebrains was measured spectrophotofluorimetrically within 24 h[10], results were analysed statistically by Student's *t* test.

In agreement with previous results[11–14], we found that LSD and N,N-dimethyltryptamine produce a significant ($P < 0.05$) increase in the serotonin in the rat brain and a decrease in the 5-HIAA (Figs. 1 and 2). Both drugs significantly ($P < 0.05$) reduced the increase in forebrain 5-HIAA regularly seen in untreated animals after raphé stimulation. In stimulated rats, however, LSD produced a small but significant ($P < 0.05$) decrease in the forebrain concentration of serotonin (Fig. 1), while there was an increase in this compound ($P < 0.025$) in animals pretreated with DMT (Fig. 2). DMT interacted more consistently with the induced increase in forebrain 5-HIAA than LSD. Our results obtained with LSD do not agree with data reported by Kostowski and Giacalone[15].

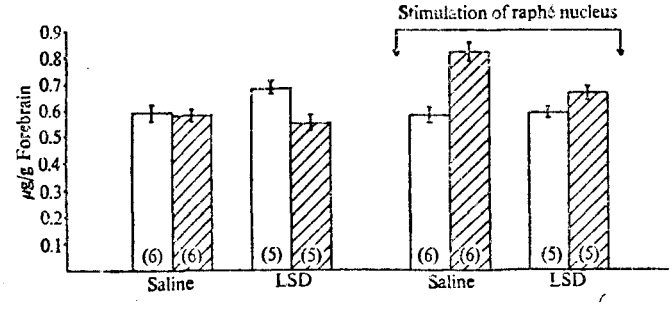

Fig. 1 Concentrations of serotonin (white columns) and 5-HIAA (hatched columns) in the forebrain with and without electrical stimulation of mid-brain raphé in control (saline) and LSD-pretreated rats. Values shown are the means (±s.e.) of five and/or six determinations (the exact numbers of animals are shown on the corresponding columns).

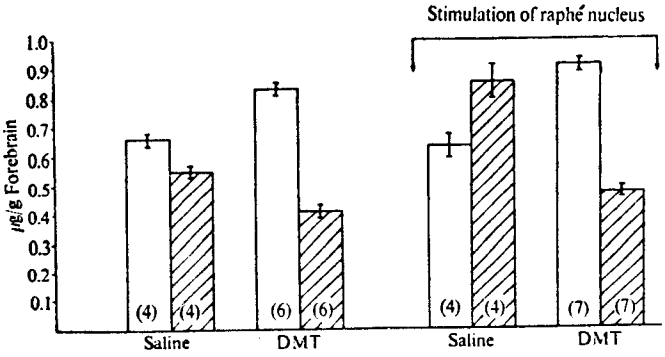

Fig. 2 Concentrations of serotonin (white columns) and 5-HIAA (hatched columns) in the forebrain with and without electrical stimulation of mid-brain raphé in control (saline) and DMT-pretreated rats. Values shown are the means (±s.e.) of four, six and/or seven determinations (the exact numbers of rats are shown on the corresponding columns).

Although the effects of LSD and DMT on brain serotonin metabolism[11–14] and on the activity of serotonin-containing neurones in raphé nuclei[8] are similar, the actions of the drugs on stimulation-induced serotonin release differed in our experiments; LSD decreased the concentration of serotonin in the forebrain, while DMT increased it. As to the possible mechanism of interaction of LSD and DMT with the altered concentrations of serotonin and 5-HIAA in the forebrain caused by raphé stimulation, several factors such as release, uptake, storage, binding, monoamine oxidase activity, drug interference with specific receptors and inhibition of neuronal activity have to be considered. Diaz *et al.*[13] found that LSD slowed the turnover of serotonin in the brain and suggested that this was a consequence of a blockade of serotonin release similar to that reported for noradrenaline in peripheral sympathetic neurones[16]. Our experiments suggest that LSD has an inhibitory effect on biosynthesis and/or the release of serotonin from nerve terminals, which would lead to a decrease in brain serotonin turnover and inhibition of serotonin-containing neurones. This hypothesis is supported by the finding that LSD blocks the release of ^{3}H-serotonin induced by electrical stimulation of brain slices[17]. As for DMT, it is believed to be a potent inhibitor of the uptake of serotonin and the effect of raphé stimulation on the serotonin and 5-HIAA in the rat forebrain might be explained on this basis. If the serotonin released by stimulation were not taken up and metabolized by monoamine oxidase, this would account for the observed increase in the forebrain serotonin content. Further, DMT may interfere with the release of serotonin.

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1 Dahlström, A., and Fuxe, K., *Acta Physiol. Scand.*, **62**, Suppl. 232, 1 (1964).
2 Aghajanian, G. K., Rosecrans, J. A., and Sheard, M. H., *Science*, **156**, 403 (1967).
3 Sheard, M. H., and Aghajanian, G. K., *J. Pharmacol. Exp. Ther.*, **163**, 425 (1968).
4 Eccleston, D., Padjen, A., and Randić, M., *J. Physiol.*, **201**, 22P (1969).
5 Kostowski, W., Giacalone, E., Garattini, S., and Valzelli, L., *Europ. J. Pharmacol.*, **7**, 170 (1969).
6 Padjen, A., and Randić, M., *Brit. J. Pharmacol.*, **39**, 1 (1970).
7 Aghajanian, G. K., Foote, W. E., and Sheard, M. H., *Science*, **161**, 706 (1968).
8 Aghajanian, G. K., Foote, W. E., and Sheard, M. H., *J. Pharmac. Exp. Ther.*, **171**, 178 (1970).
9 König, J. F. R., and Klippel, R. A., *The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem* (Williams and Wilkins, Baltimore, 1963).
10 Ashcroft, G. W., and Sharman, D. F., *Brit. J. Pharmacol.*, **19**, 153 (1962).
11 Freedman, D. X., *J. Pharmacol. Exp. Ther.*, **134**, 160 (1961).
12 Rosecrans, J. A., Lovell, R. A., and Freedman, D. X., *Biochem. Pharmacol.*, **16**, 2011 (1967).
13 Diaz, P. M., Ngai, S. H., and Costa, E., in *Advances in Pharmacology* (edit. by Costa, E., Garattini, S., Sandler, M., and Shore, P. A.) (Academic Press, New York, 1968).
14 Freedman, D. X., Gottlieb, R., and Lovell, R. A., *Biochem. Pharmacol.*, **19**, 1181 (1970).
15 Kostowski, W., and Giacalone, E., in *The Present Status of Psychotropic Drugs* (edit. by Cerletti, A., and Bové, F. J.) (Excerpta Medica Foundation, Amsterdam, 1969).
16 Reid, W. D., Volicer, L., and Brodie, B. B., *Fed. Proc.*, **26**, 463 (1967).
17 Chase, T. N., Breese, G. R., and Kopin, I. J., *Science*, **157**, 1461 (1967).

3-O-Methyldopa, a New Precursor of Dopamine

3-METHOXY-4-hydroxyphenylalanine (3-O-methyldopa) is a major metabolite of L-3,4-dihydroxyphenylalanine (L-dopa) in animals and man[1–4] and when labelled it easily penetrates from the blood into other tissues, for example the brain and heart, where it has a biological half life of 12–13 h, which is considerably longer than that of L-dopa, which is about 30 min. The bulk of the urinary radioactivity excreted within 24 h consists of homovanillic acid (3-methoxy-4-hydroxyphenylacetic acid) and 3-methoxy-4-hydroxyphenyllactic acid[5].

We have identified and measured other minor metabolites of L-3-O-methyldopa in the urine, and investigated the effect of enzyme inhibitors on the metabolism of this amino-acid in the brain. We wish to report evidence that L-3-O-methyldopa is partially demethylated in the organism.

Rats of both sexes were given L-2-^{14}C-3-O-methyldopa (specific activity 193 μCi/mg) intraperitoneally. Some of the animals were pretreated with various inhibitors of catecholamine metabolism. The urine was collected for 4 h and incubated with 'Glusulase'. Brain homogenates were deproteinized and submitted to column chromatography to separate amino-acids, catecholamines and phenolcarboxylic acids, which were isolated by paper chromatography and measured for radioactivity in a liquid scintillation counter as described before[3,5].

About 6% of the administered radioactivity was found in the urine. Table 1 shows that about 8.5% of the excreted radioactivity was in the dihydroxylated metabolites dopa, dopamine and 3,4-dihydroxyphenylacetic acid. About 74% of the metabolites, however, consisted of 3-methoxy-4-hydroxy-

Table 1 Radioactive Metabolites of L-2-^{14}C-3-O-Methyldopa in the Urine

Demethylated metabolites	
Dopa	0.62±0.01
Dopamine	3.35±0.06
3,4-Dihydroxyphenylacetic acid	4.64±0.68
Methylated metabolites	
3-O-Methyldopa	4.68±0.01
3-Methoxytyramine	2.05±0.06
Homovanillic acid	45.45±1.25
3-Methoxy-4-hydroxy-phenyllactic acid	26.37±1.32
Unidentified acidic metabolites	12.27±0.72

Metabolites were collected 4 h after intraperitoneal administration of 1 mg/kg L-2-^{14}C-3-O-methyldopa to rats. The values are expressed in percentage of total radioactivity found in the urine, and represent averages with s.e. of two to eight experiments each with pooled urines from six rats. The total radioactivity excreted in the urine in the first 4 h of the experiment amounted to 6.4±0.8% of the total injected radioactivity.