Biochemical Pharmacology, Vol. 26, pp. 1450–1451. Pergamon Press, 1977. Printed in Great Britain.

Evidence against the view that the central actions of polyamines are indirectly mediated

(*Received* 10 *November* 1976; *accepted* 9 *February* 1977)

When high doses of spermidine or spermine are administered by intraperitoneal or intravenous injection they produce sedation, hypomotility, hypothermia [1] and hyperglycaemia [2]. These effects are also produced when much smaller amounts of these polyamines are injected into the cerebral ventricles [3] and it is, therefore, likely that they are the result of an action on the central nervous system. It has recently been suggested that the central effects produced by intraperitoneal injection of high doses of polyamines may be mediated indirectly through the agency of some other neurohumoral substance such as noradrenaline or 5-hydroxytryptamine [4]. Furthermore, the finding that, in suitable concentrations, spermidine and spermine can inhibit or activate cholinesterase [5] could indicate an action mediated through a cholinergic system. The present experiments were undertaken in order to substantiate or refute the suggestion that the central actions of polyamines are indirectly mediated.

Female Wistar rats weighing 120–130 g were injected by the intraperitoneal route with a solution of sodium chloride (0.9%), spermine tetrahydrochloride (equivalent to 30 mg base per kg) or spermidine trihydrochloride (equivalent to 100 mg base per kg) in a dose volume of 0.5 ml/100 g. The animals were killed 45 min after the injection. When the acetylcholine or γ-aminobutyric acid content of the brain was to be determined the animals were killed by rapid freezing in liquid nitrogen and the brain was removed while it was still frozen. In all other experiments the animals were killed by decapitation and the brain was removed at room temperature.

Whole brain acetylcholine content was determined by bioassay on the frog rectus abdominis preparation [6]. Noradrenaline and dopamine were determined by fluorometric assay following separation on activated alumina [7, 8]. The solvent extraction procedure devised by Snyder, Axelrod and Zweig [9] was used to determine 5-hydroxytryptamine. γ-Aminobutyric acid was isolated by paper chromatography and estimated by spectrophotometry [10] and histamine in both brain and a 2.0 ml sample of blood was determined by fluorometry after ion-exchange chromatography on Bio-Rex 63 [11]. The recovery in each assay procedure was determined by adding known quantities of the respective substance to brain homogenate. With the exception of 5-hydroxytryptamine, which was recovered in a yield of 88 per cent, recovery was complete. Values for the 5-hydroxytryptamine content of brain were corrected to take recovery into account.

The volume of blood contained in the excised brain was determined by isotope dilution using 0.1 ml ^{125}I human serum albumin injection B.P. administered by intravenous injection. The animals were killed by decapitation after 10 min [12] and the radioactivity of a 0.1 ml sample of blood and of the excised brain was determined.

Following the administration of spermine or spermidine no statistically significant change was found in the concentration of acetylcholine, noradrenaline, dopamine, 5-hydroxytryptamine or γ-aminobutyric acid in the brain (Table 1). Values for control animals are in good agreement with accepted literature values for normal rat brain.

In accordance with previously reported findings [1] both spermidine and spermine produced a large increase in the histamine content of the blood of the animals. The blood histamine content of animals which had received sodium chloride solution was 0.37 ± 0.01 (S.E.M.) nmol/ml whereas that of animals given spermine was 0.72 ± 0.09 nmol/ml and that of spermidine injected animals 2.66 ± 0.25 nmol/ml. The mean blood content of the excised brains was 4.82 ± 0.96 (S.E.M.) ml/100 g. The calculated mean contribution to brain histamine content made by histamine present in blood was 0.018 ± 0.001 (S.E.M.) nmol/g in saline injected animals, 0.034 ± 0.010 nmol/g in spermine injected animals and 0.124 ± 0.027 nmol/g in those given spermidine. After correction for this contribution, no statistically significant change in brain tissue histamine content was detected (Table 1).

The present experiments support the view that the effects on the central nervous system produced by spermine and spermidine are mediated directly rather than through the agency of any of the several substances estimated whose role in neurotransmission is accepted or canvassed. Since the polyamines are present in high concentrations in the brain [13] there is indeed no reason to presuppose that they should act through such an agency. It must be admitted that the present experiments are not absolutely conclusive since it is, for example, possible for the turnover of a substance to be changed without significant alteration of the whole brain content. Similarly, a localised change in the concentration of a substance in a discrete region of the brain could also occur without the total brain con-

Table 1. The concentration of some neurohumoral substances in rat brain following the administration of spermine or spermidine

Substance assayed	Treatment		
	Control (0.9% NaCl)	Spermine (30 mg/kg)	Spermidine (100 mg/kg)
Acetylcholine	17.3 ± 5.1	18.8 ± 3.6	16.2 ± 3.9
Noradrenaline	2.13 ± 0.23	1.95 ± 0.29	2.01 ± 0.35
Dopamine	5.75 ± 0.45	4.96 ± 0.78	4.84 ± 0.65
5-Hydroxytryptamine	2.78 ± 0.40	2.50 ± 0.34	2.50 ± 0.51
γ-Aminobutyric acid	1602 ± 340	1582 ± 243	1515 ± 311
Histamine	0.557 ± 0.047	0.619 ± 0.068	0.578 ± 0.079

Values are the mean content ± S.E.M. for 5 animals expressed as nmol/g wet wt.

tent being markedly affected. Under the present experimental conditions the likelihood of such events is remote but the observation in the present investigation that changes in blood histamine concentration can make a significant contribution to estimates of brain histamine content, illustrates the need for caution in the interpretation of experimental findings.

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Biochemical Pharmacology, Vol. 26, pp. 1451–1452. Pergamon Press, 1977. Printed in Great Britain.

Absence of increased carnitine acetyltransferase activity in the liver with proliferation of smooth endoplasmic reticulum

(*Received* 12 *October* 1976; *accepted* 17 *December* 1976)

Carnitine acetyltransferase (CAT; EC 2.3.1.7) of rat liver has been shown by Markwell *et al.* [1, 2] to be distributed among the mitochondrial, peroxisomal and microsomal fractions. Previous work from our laboratory has demonstrated that the marked increase in CAT activity in the livers of rats and mice treated with several hypolipidemic drugs is, to a large extent, due to the striking increase in peroxisome population [3–5]. As the subcellular distribution of CAT is heterogenous in nature, and since the drugs that induce peroxisome proliferation also cause a concomitant increase in smooth endoplasmic reticulum (SER) [5–7], the possibility that SER may also contribute to the CAT increase could not be ruled out. The present study was undertaken to ascertain if compounds capable of inducing proliferation of SER can cause an increase in CAT activity in the liver.

Male F-344 rats (Simonson Labs, Inc., Gilroy, CA) were treated for 1 week with a peroxisome proliferating agent, clofibrate (ethyl-α-*p*-chlorophenoxyisobutyrate) [6, 8], or with drugs known to induce the proliferation of hepatic SER [9–11]. These are: phenobarbital (Winthrop Labs, New York, NY), allylisopropylacetamide (AIA; Hoffmann-LaRoche, Inc., Nutley, NJ) and [1-(*o*-chloraphenyl)-1-(*p*-chlorophenyl), 2,2-dichloroethanc] (*o,p*-DDD; Aldrich

Table 1. Effect of compounds that induce proliferation of hepatic peroxisomes or smooth endoplasmic reticulum on liver weight and carnitine acetyltransferase activity in male F-344 rats

Treatment*	No. of animals	Liver wt (g/100 g body wt)	Hepatic CAT activity (units/mg protein)
Control diet	4	4.52 ± 0.06†	3.3 ± 0.4
Clofibrate (100 mg/kg; gavage 2 times daily)	4	6.13 ± 0.38‡	57.8 ± 6.2‡
Phenobarbital (100 mg/kg; i.p. once daily)	5	4.98 ± 0.04‡	1.2 ± 0.3§
Allylisopropylacetamide (200 mg/kg; S.C.; 2 times daily)	5	6.07 ± 0.24‡	4.2 ± 1.4
o,p-DDD (200 mg/kg; i.p. once daily)	5	4.15 ± 0.10§	3.2 ± 0.9

* All animals were treated for 7 consecutive days. Most received a single dose, except for clofibrate and AIA groups which received 2 doses/day.
† Values are expressed as mean ± S. E.
‡ Significantly different from control, $P < 0.001$.
§ Significantly different from control, $P < 0.05$.