Biochemical Pharmacology, 1963, Vol. 12, pp. 1279–1282. Pergamon Press Ltd., Printed in Great Britain.

EFFECTS OF MINOR TRANQUILLIZERS ON BRAIN PHOSPHATE LEVELS *IN VIVO*

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(*Received* 31 *May* 1963; *accepted* 27 *June* 1963)

Abstract—Investigation of the effects of certain diphenylmethane derivatives, chlordiazepoxide, meprobamate and 1-(2′-tetrahydrofuryl)-1-ethylamino-2-phenylethane (WIN 19583-4) upon levels of adenine nucleotides, phosphocreatine and inorganic phosphate in the rat brain *in vivo* indicates that behavioural depression is associated with a fall in adenosine triphosphate and a rise in adenosine diphosphate levels. The behavioural stimulants had the reverse effects. Changes in the adenine nucleotide levels appear to be correlated with effects upon behaviour.

STUDIES upon amphetamine-like drugs, antidepressives and major tranquillizers have shown behavioural stimulation and depression in rats to be associated respectively with raised and lowered brain levels of adenosine triphosphate (ATP) and with correspondingly reduced and increased levels of adenosine diphosphate (ADP).[1–4] More recent investigations have shown that psychotomimetics have qualitatively similar effects to other behavioural stimulants (Lewis, Ritchie and Van Petten, unpublished observations). The experiments reported in this paper extend these studies to the investigation of a group of minor tranquillizers, including the diphenylmethane derivatives azacyclonol and hydroxyzine, together with chlordiazepoxide and meprobamate. The two former compounds have been compared with two chemically related diphenylmethane derivatives which cause behavioural stimulation, namely, benactyzine and pipradrol and the β-phenyl ethylamine derivative 1-(2′-tetrahydrofuryl)-1-ethylamino-2-phenylethane (WIN-19583-4).

METHODS

Male Wistar rats weighing from 70–95 g were placed in groups of two, one serving as control, the other receiving the drug. The order of treatment was randomized, using a table of random numbers. Azacyclonol, benactyzine, hydroxyzine, pipradrol, chlordiazepoxide and WIN-19583-4 were used as the hydrochlorides. With the exception of meprobamate which was dissolved in the minimum quantity of absolute ethanol and the solution adjusted to volume with deionized water, all drug solutions were made in 0·9 per cent w/v sodium chloride and sterilized by filtration. Solutions of chlordiazepoxide were always freshly made, and were not sterilized. Matching control solutions were used throughout the experiments. Injections were made intraperitoneally. Apart from series 5, 8 and 10, in which it was 1 ml/100 g body weight, the volume injected was 0·2 ml/100 g body weight. Killing, dissection, extraction of the brain and assays for adenine nucleotides, phosphocreatine and inorganic phosphate

were as described by Lewis and Van Petten (1962).[1] The results were tested for significance, using an analysis of variance.

RESULTS

The results are summarized in Tables 1 and 2, from which it can be seen that there was no significant effect on levels of adenosine monophosphate (AMP), inorganic phosphate (except series 9), phosphocreatine (except series 10 and 11) or the total adenine nucleotides (AMP + ADP + ATP; except series 1 and 8).

Apart from meprobamate, the minor tranquillizers all lowered ATP and raised ADP levels, causing the ATP/ADP ratio to fall. Even the larger dose of meprobamate (200 mg/kg), which caused complete paralysis, was ineffective in changing the adenine nucleotide levels significantly. Chlordiazepoxide (60 mg/kg) caused a highly significant fall in the ATP/ADP ratio. At this dose level, the animals were both depressed and paralysed. The hydroxyzine-treated animals were drowsy and depressed, their movements were slowed and they would remain in the same posture if left undisturbed. The lower dose of azacyclonol did not noticeably depress the animals, but 100 mg/kg produced marked depression. Pipradrol at the higher dose level and benactyzine both caused increased alertness and motor activity; WIN-19583-4 caused no noticeable changes in behaviour.

Pipradrol did not cause a significant rise in ATP until 40 mg/kg were used. At this dose level the animals were stimulated. They were also stimulated at 20 mg/kg, but this dose did not cause significant changes in adenine nucleotide levels. The behavioural stimulation associated with 4 mg/kg of benactyzine was accompanied by a highly significant rise in ATP and a highly significant fall in ADP. These changes caused a significant rise in the ATP/ADP ratio.

Azacyclonol (α-(4-piperidyl)benzhydrol hydrochloride) and pipradrol (α-(2-piperidyl) benzhydrol hydrochloride) are isomeric so that their opposing effects on behaviour and on adenine nucleotide levels are of interest. WIN-19583-4 caused no changes in the behaviour or the adenine nucleotide levels in the dose used.

DISCUSSION

The results indicate that behavioural depression or sedation are associated respectively with a lowered ATP and a reduced ATP/ADP ratio. The converse holds true for the behavioural stimulants tested. The demonstration of this relationship among minor tranquillizers and some chemically related stimulants confirms a relationship already shown.[1–4] Of the drugs tested, however, only chlordiazepoxide and hydroxyzine had marked behavioural depressant activity and the effects of the former were complicated by the simultaneous occurrence of muscular paralysis. The doses of chlordiazepoxide used were of the same order as those shown by Randall *et al.*[5] to cause depression of spontaneous motor activity in rats. It is noteworthy that animals paralysed by the higher dose of meprobamate showed no significant changes in adenine nucleotides, yet the chlordiazepoxide and meprobamate-treated animals appeared equally paralysed. This difference can be explained by assuming that, at the doses used, meprobamate acts only on the spinal cord but chlordiazepoxide also acts on the brain in a manner similar to other tranquillizers. Hydroxyzine and chlordiazepoxide were apparently approximately equipotent in their effects on adenine nucleotides, but the former, presumably due to its lack of inter-neuronal blocking activity,

TABLE 1. *In vivo* EFFECTS OF BENACTYZINE, HYDROXYZINE, AZACYCLONOL, AND PIPRADROL ON THE ADENINE NUCLEOTIDE, PHOSPHOCREATINE AND INORGANIC PHOSPHATE CONCENTRATIONS IN THE RAT BRAIN.

Series	Treatment	Dose (mg/kg)	Time after treatment (hr)	Concentrations in μmole/g frozen brain: Inorganic phosphate	Phosphocreatine	AMP	ADP	ATP	TOTAL AMP+ADP+ATP	Ratio ATP/ADP
1	Control	—		6·06 ±0·52	2·90 ±0·19	0·70 ±0·03	0·92 ±0·04	2·26 ±0·07	3·89 ±0·09	2·47 ±0·08
	Benactyzine	4	1½	5·72 ±0·49	3·12 ±0·19	0·70 ±0·03	0·71 ±0·07‡	2·51 ±0·06‡	3·93 ±0·09†	3·92 ±0·53†
2	Control	—		6·25 ±0·74	3·53 ±0·12	0·90 ±0·05	0·89 ±0·06	2·55 ±0·07	4·33 ±0·11	2·97 ±0·19
	Hydroxyzine	30	1½	7·67 ±0·97	3·42 ±0·18	0·97 ±0·05	1·13 ±0·08‡	2·37 ±0·09	4·46 ±0·11	2·23 ±0·21†
3	Control	—		6·27 ±0·89	3·16 ±0·11	0·96 ±0·07	0·87 ±0·06	2·63 ±0·07	4·46 ±0·12	3·15 ±0·23
	Hydroxyzine	60	1½	5·69 ±0·70	3·19 ±0·10	1·05 ±0·05	1·10 ±0·05‡	2·38 ±0·06†	4·53 ±0·08	2·19 ±0·10‡
4	Control	—		5·73 ±0·42	3·38 ±0·15	1·07 ±0·08	1·13 ±0·05	2·40 ±0·11	4·60 ±0·06	2·21 ±0·22
	Azacyclonol	30	3	6·79 ±1·05*	3·29 ±0·09	1·10 ±0·07	1·15 ±0·09	2·29 ±0·08	4·54 ±0·12	2·14 ±0·24
5	Control			5·43 ±0·45	3·19 ±0·09	1·15 ±0·06	0·97 ±0·05	2·67 ±0·10	4·80 ±0·16	2·79 ±0·14
	Azacyclonol	100	3	5·73 ±0·47	3·47 ±0·18	1·01 ±0·04	1·14 ±0·08	2·43 ±0·08†	4·59 ±0·12	2·26 ±0·24
6	Control	—		6·13 ±0·40	3·20 ±0·16	1·08 ±0·06	1·01 ±0·05	2·54 ±0·09	4·63 ±0·07	2·59 ±0·17
	Pipadrol	10	1	5·40 ±0·26	3·45 ±0·14	1·02 ±0·06	0·83 ±0·06	2·73 ±0·07	4·58 ±0·08	3·51 ±0·38
7	Control	—		5·24 ±0·35	3·09 ±0·13	1·11 ±0·06	0·93 ±0·06	2·27 ±0·09	4·31 ±0·11	2·57 ±0·21
	Pipradrol	20	1	5·53 ±0·47*	3·04 ±0·17	1·08 ±0·05	0·88 ±0·04	2·43 ±0·07	4·39 ±0·07	2·80 ±0·15
8	Control	—		6·24 ±0·77	3·15 ±0·10	1·03 ±0·04	0·96 ±0·06	2·31 ±0·13	4·31 ±0·15	2·55 ±0·30
	Pipradrol	40	1	5·79 ±0·44	3·02 ±0·16	1·13 ±0·06	0·88 ±0·06	2·66 ±0·07†	4·70 ±0·12†	3·19 ±0·28

All values are the means ±(S.E.) of determination on ten rats.
Significance of difference from control: * one missing value calculated † 0·05 > P > 0·01 ‡ 0·01 > P > 0·001.

TABLE 2. *In vivo* EFFECTS OF MEPROBAMATE, CHLORDIAZEPOXIDE AND 1-(2′- TETRAHYDROFURYL)-1-ETHYLAMINO-2-PHENYLETHANE (WIN-19583-4) ON THE ADENINE NUCLEOTIDE, INORGANIC PHOSPHATE AND PHOSPHOCREATINE CONCENTRATIONS IN THE RAT BRAIN.

Series	Treatment	Dose (mg/kg)	Concentrations in μmole/g frozen brain: Inorganic phosphate	Phosphocreatine	AMP	ADP	ATP	TOTAL AMP+ADP+ATP	Ratio ATP/ADP
9	Control	—	4·91 ±0·19	3·01 ±0·17	0·84 ±0·05	0·74 ±0·07	2·23 ±0·09	3·82 ±0·16	3·29 ±0·39
	Meprobamate	50	6·28 ±0·55†	2·90 ±0·18	0·80 ±0·06	0·90 ±0·08	2·21 ±0·08	3·91 ±0·16	2·61 ±0·19
10	Control	—	5·41 ±0·95	3·11 ±0·13	1·03 ±0·05	0·94 ±0·06	2·51 ±0·10	4·48 ±0·12	2·78 ±0·22
	Meprobamate	200	4·96 ±0·32	3·55 ±0·10†	0·95 ±0·06	0·92 ±0·10	2·54 ±0·07	4·40 ±0·20	3·01 ±0·27
11	Control	—	6·51 ±0·73	3·10 ±0·22	0·96 ±0·09	0·82 ±0·04	2·61 ±0·08	4·40 ±0·13	3·26 ±0·22
	Chlordiazepoxide	60	5·58 ±0·73*	3·58 ±0·21‡	0·87 ±0·09	1·14 ±0·06§	2·38 ±0·06	4·38 ±0·14	2·13 ±0·11§
12	Control	—	5·56 ±0·53	2·86 ±0·13	1·01 ±0·08	0·85 ±0·06	2·31 ±0·12	4·18 ±0·10	2·93 ±0·33
	WIN-19583-4	10	5·42 ±0·52	2·88 ±0·10	1·06 ±0·05	1·03 ±0·09	2·14 ±0·07	4·23 ±0·10	2·26 ±0·27

All values are the means (±S.E.) of determination on ten rats made 3 hr after injection of the drug or control solution.
Significance of difference from control: * one missing value calculated † 0·05 > P > 0·01 ‡ 0·01 > P > 0·001 § 0·001 > P.

produced no paralysis. Azacyclonol only caused significant changes in adenine nucleotides and behavioural depression at a dose of 100 mg/kg, which supports the view that this is a compound of low potency.[6]

The observations reported in this paper and those recorded elsewhere[1-4] point to a fairly consistent association between behavioural changes among tranquillizers and anti-depressives and alterations in brain adenine nucleotide levels. As far as we have been able to ascertain, these effects are more consistent than other properties possessed by these compounds. Thus where these have been investigated, there appear to be no effects upon brain amine levels.[6,7] Benactyzine and hydroxyzine both possess atropine-like peripheral activity and the latter is an antihistamine and antiserotonin of moderate potency while the former inhibits mono-amine oxidase in a non-competitive fashion.[8] Both benactyzine and meprobamate inhibit oxidative phosphorylation in preparations of the hypothalamus of the rat, showing a selective action upon this region.[9]

McIlwain (1962) has suggested that depressants diminish the movements of ions,[10] and it is known that energy for ion fluxes is supplied by ATP breakdown. It is therefore possible that the depressants which we have tested may reduce the amount of energy available by depressing ATP synthesis, or by depressing the efficiency with which the energy yielded is utilized. It is also possible that they increase ATP-ase activity. The stimulants act in the converse fashion.

The doses used in these experiments are much higher than the average single dose used clinically. It would not, therefore, be justifiable to assume that the changes recorded in adenine nucleotide levels in rats occur in man, and are necessarily responsible for the clinical effects. There are, however, reports of the prolonged use of relatively high doses of these drugs, for example, benactyzine has been given at a dose of 60 mg/day. Up to 500 mg/day of hydroxyzine has been used in children[11] and Sherrod[6] records that doses of 800 mg to 1 g have been used clinically without adverse effects. Niswander and Holt have used up to 400 to 800 mg daily of azacyclonol.[12] Our results may therefore be relevant to the clinical effects recorded at such dose levels.

Acknowledgements—We wish to thank Dr. R. A. Robb for his advice and help with the statistical methods used and Dr. I. R. W. Lominski for sterilizing the solutions. We thank Dr. J. Goulton, Pfizer Limited (Hydroxyzine), Dr. D. Mansel-Jones, Merrell-National (Laboratories) Ltd. (Azacyclonol and Pipradrol), Dr. J. Wynn Jones, Roche Products Ltd. (Chlordiazepoxide), Dr. B. W. Cromie, John Wyeth & Brother, Ltd., (Meprobamate), Mr. E. M. Bavin, Smith and Nephew Research Ltd. (Benactyzine) and Dr. J. Rae, Bayer Products (WIN. 19583-4) for their generous gifts of drugs.

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