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EFFECTS OF PSYCHOPHARMACOLOGICAL AGENTS ON BRAIN METABOLISM—II.

INFLUENCE OF IMIPRAMINE AND PROTHIADENE ON THE FREE NUCLEOTIDE LEVEL OF RAT BRAIN

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Abstract—The effect of a single intraperitoneal dose of the antidepressive drugs, Imipramine and Prothiadene, was studied on the level of free nucleotides in the rat brain. Imipramine and Prothiadene have no marked influence on the levels of adenine nucleotides after 1 hr but after 3 hr action they increase ATP levels, and lower ADP; Prothiadene also lowers the level of AMP. Hence the molar ratios ATP to AMP and ATP to ADP rise significantly. Both drugs elevated UTP levels in rat brain 1 hr after administration, and this level remained high also after action of these substances for 3 hr.

EXTENSIVE pharmacological testing of Prothiadene carried out in recent years[1, 8] has shown this substance to have a marked effect on the central nervous system. Due to the nature of its pharmacological action as well as on the basis of clinical experience[15] it was possible to recognise this substance as an effective antidepressive with an antireserpine and antihistamine effect.

In experiments on rats we studied the influence of Prothiadene on levels of free nucleotides in the brain and compared its action with another antidepressive drug, Imipramine. It was found in experiments *in vitro* that both of these substances influenced some parts of protein and lipid metabolism to the same extent.[13]

EXPERIMENTAL

Experiments were carried out on white male rats weighing 140–160 g and fed on standard Larsen diet.

Imipramine and Prothiadene (which were kindly provided by Dr. Protiva from the Research Institute for Pharmacy and Biochemistry in Prague) were dissolved in 0·9% saline solution at a concentration of 25 mg/ml and administered intraperitoneally in doses of 50 mg/kg body weight. The control group received an equivalent volume of 0·9% saline solution.

Animals were sacrificed by submerging their heads into a vessel filled with liquid air. The frozen heads were opened and the brain tissue was pulverized under continuous cooling with liquid air and was extracted in a 5 fold volume of a 10 per cent solution of trichloracetic acid in acetone by homogenization in a Potter, Elvehjem homogenizer cooled in a mixture of solid CO_2-acetone according to the procedure of Minard and Davis.[9, 10] The homogenate was warmed to freezing point and an equal amount of cold 10% aqueous solution of trichloracetic acid was added. The acetone was removed

by evaporation in a rotary vacuum evaporator at 25° and the homogenate then centrifuged at 3000 *g* for 10 min. The trichloracetic-acid was removed from the clear supernatant by extracting with ethylether several times. The neutral aqueous phase was then used to estimate free nucleotides.

The free nucleotides were separated chromatographically on a 1 × 8, 200–400 mesh Dowex-formate column, 1 cm in diameter and 15 cm in length. The elution was accomplished using a gradient of formic acid and ammonium formate[12] at a flow rate of 1·5 ml/min. 5 ml samples were collected using an automatic fraction collector and their optical density measured at 260 μm. The analysis of the fractions obtained, as well as the calculation of nucleotide quantities, was made as described in our previous work.[11]

RESULTS

The chromatograms obtained of nucleotides present in the trichloracetic acid extract of rat brain enabled us to evaluate the quantities of mono-, di- and triphosphoric esters of adenosine, uridine and guanosine, NAD, ADPR and CMP as well as UDPG, UDPAG and UDP*x*, which are grouped together in the results as UDPC*o**. (Fig. 1).

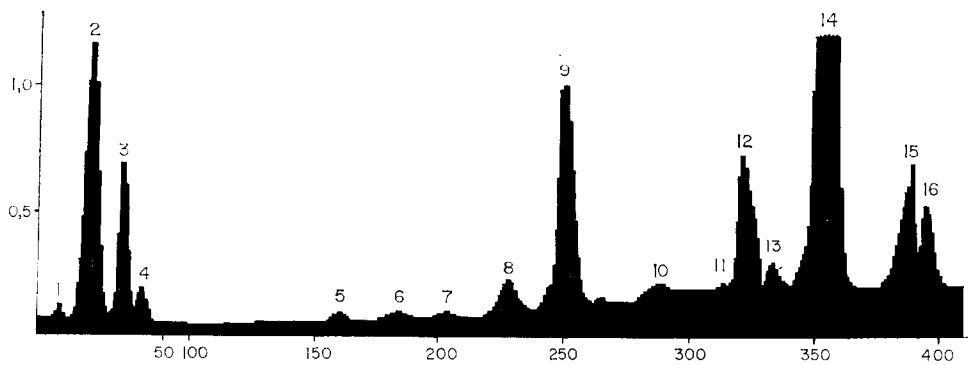

FIG. 1. Separation of free nucleotides in rat brain on 1 × 8 Dowex (formate) column by means of gradient of formic acid and ammonium formate. Arrangement of gradient: specimens 1–93 water and 0·5M formic acid; 94–186, 0·5M formic acid and 1 M formic acid; 187–280, 1M formic acid and 4M formic acid; 281–330, 4M formic acid and 4M formic acid + 0·2M ammonium formate; 331 –end of elution, 4M formic acid +0·2M ammonium formate and 0·4M formic acid + 0·6M ammonium formate. Ordinate: optical density of specimens; abscissa: number of specimens. Peaks: 1-CMP, 2-NAD, 3-unknown, 4-AMP, 5-GMP, 6-IMP, 7-UMP, 8-ADPR, 9-ADP, 10-UDPAG, 11-UDPX, 12-UDPG, 13-GDP, 14-UDP and ATP, 15-GTP 16-UTP.

The influence of Imipramine and Prothiadene on the amounts of free nucleotides in rat brain was checked at 1 and 3 hr after administration. The quantities of free nucleotides found in rat brain after administering both substances are shown in Table 1.

* The following abbreviations were used: AMP, ADP, ATP-adenosine mono-, di-, and tri-phosphate; GMP, GDP, GTP-guanosine mono-, di- and triphosphate; UMP, UDP, UTP-uridine mono-, di- and triphosphate; CMP-cytidine monophosphate; UDPAG -uridine diphosphoacetylglucosamine; UDPG-uridine diphosphoglucose; ADPR-adenosine diphosphoribose; NAD-nicotinamide adenine dinucleotide; IMP-inosine monophosphate.

TABLE 1. FREE NUCLEOTIDE CONTENT OF RAT BRAIN AFTER ADMINISTRATION OF IMIPRAMINE AND PROTHIADENE

Nucleotides	Imipramine		Prothiadene		Control animals
	Time after administration 1 hr	3 hr	1 hr	3 hr	
AMP	5·8 ± 0·69	3·5 ± 0·88	4·5 ± 0·61	2·8 ± 0·27	5·5 ± 0·73
ADP	32·5 ± 3·28	26·0 ± 3·21	30·4 ± 1·35	25·3 ± 1·09	33·8 ± 1·76
ATP	148·8 ± 5·36	183·3 ± 8·62	167·2 ± 6·63	192·5 ± 2·47	157·5 ± 8·35
GMP	2·3 ± 0·55	1·9 ± 0·67	3·1 ± 0·1	2·0 ± 0·27	2·7 ± 0·5
GDP	4·2 ± 1·47	4·9 ± 1·12	5·7 ± 0·81	4·3 ± 0·93	5·1 ± 0·78
UMP	2·2 ± 0·63	2·7 ± 1·1	2·5 ± 0·45	3·7 ± 0·81	3·9 ± 0·52
UDP	7·5 ± 0·45	7·0 ± 0·18	7·1 ± 0·21	6·3 ± 0·73	7·4 ± 0·4
UTP	13·7 ± 0·76	15·6 ± 1·49	16·9 ± 1·05	15·7 ± 2·24	11·1 ± 0·62
UDPCo	32·6 ± 1·17	32·5 ± 2·14	31·3 ± 1·25	33·8 ± 2·46	35·1 ± 1·71
CMP	1·2 ± 0·58	3·2 ± 1·55	2·8 ± 0·47	2·8 ± 0·93	3·7 ± 0·57
NAD	21·6 ± 1·45	24·9 ± 0·61	23·7 ± 1·25	22·3 ± 1·01	22·4 ± 0·98
ADPR	5·1 ± 0·58	4·8 ± 0·32	5·8 ± 0·4	5·8 ± 1·08	5·8 ± 0·49
IMP	3·8 ± 0·13	4·7 ± 0·45	2·7 ± 0·43	3·5 ± 0·22	5·5 ± 0·97
Animals (No)	9	9	15	12	18
Estimations (No)	3	3	5	4	6

Values are expressed as μmoles per 100 g of frozen tissue (mean and standard error of the mean).

After acting for one hr neither Imipramine nor Prothiadene influenced the quantities of most of the investigated nucleotides. The only exceptions were the uracil nucleotides, where both Imipramine and Prothiadene evoked a significant rise in the level of UTP compared with the control group: Imipramine by 23·5 per cent ($P < 0·05$) and Prothiadene by more than 50 per cent ($P < 0·001$).

The influence of both Imipramine and Prothiadene was more profound 3 hr after their application. A marked rise in the amount of UTP was found after administering Imipramine ($P < 0·02$) or Prothiadene ($P < 0·05$). The levels of individual adenine nucleotides changed markedly. After administering Prothiadene a significant drop in the quantities of AMP ($P < 0·01$) and ADP ($P < 0·05$) occurred but on the other hand a significant increase in the level of ATP ($P < 0·05$) took place in rat brain. These changes were fully reflected also in the molar ratio of individual adenine nucleotides (Fig. 2).

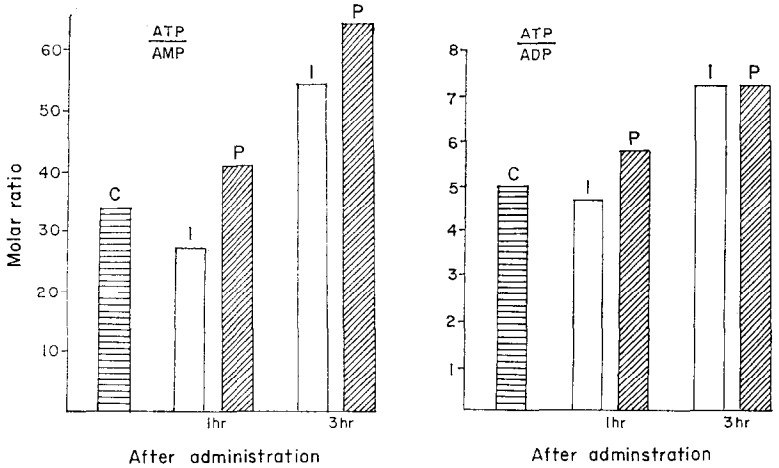

FIG. 2. Molar ratios of adenine nucleotides after administration of Prothiadene and Imipramine. C-control animals, I-Imipramine, P-Prothiadene.

In animals which were given Prothiadene a significant increase of ATP to AMP ratio ($P < 0.002$) and ATP to ADP ($P < 0.02$) was observed at 3 hr compared with control groups of animals. The ATP to AMP ratio was also significantly higher in those animals in which Prothiadene acted for only 1 hr ($P < 0.01$) compared with controls.

Three hr after administration of Imipramine the amount of AMP in rat brain was practically the same as in the control group although a certain reduction of the ADP quantity as well as an elevation of the ATP quantity did occur. These changes in the quantities of adenine nucleotides become significant if we compare the molar ratio of these nucleotides in animals after the action of Imipramine for 3 hr, when a markedly higher molar ratio ATP to ADP ($P < 0.05$) and ATP to AMP ($P < 0.05$) was found.

No quantitative changes were found for other types of nucleotides studied at either 1 or 3 hr after application. A nonsignificant increase of total adenine nucleotides, uridine nucleotides and of total free nucleotides did occur in animals 3 hr after administration of Imipramine or Prothiadene (Table 2).

TABLE 2. QUANTITIES OF INDIVIDUAL TYPES OF NUCLEOTIDES AND TOTAL AMOUNT OF FREE NUCLEOTIDES AFTER ADMINISTRATION OF IMIPRAMINE AND PROTHIADENE. (DATA AS IN TABLE 1).

Nucleotides	Imipramine		Prothiadene		Control animals
	Time after administration		Time after administration		
	1 hr	3 hr	1 hr	3 hr	
Adenine nucleotides	187·3 ±2·29	210·8 ±6·19	208·1 ±4·11	220·6 ±3·29	195·6 ±9·36
Guanine nucleotides	35·2 ±4·24	39·9 ±·82	38·2 ±0·91	39·1 ±3·24	38·3 ±1·7
Uridine nucleotides without UDPCo	23·4 ±0·97	25·3 ±1·9	26·7 ±1·17	25·5 ±2·53	22·9 ±1·61
Uridine nucleotides with UDPCo	56·1 ±1·29	57·9 ±3·49	58·8 ±2·32	59·3 ±2·56	56·1 ±3·31
Total amount of nucleotides	314·2 ±8·41	346·0 ±13·69	339·2 ±5·0	356·8 ±0·47	329·0 ±11·82

DISCUSSION

The level of adenine nucleotides has been studied in the brain of animals after administration of some psychopharmaceuticals. The greatest attention has been paid to chlorpromazine and reserpine. Chlorpromazine did not change the level of adenine nucleotides in rat brain.[2, 3] But significantly reduced the speed of ATP destruction.[9, 16] Reserpine decreased the amount of ATP in rat brain.[4, 5] Lewis and Van Petten[6, 14] described the effect of a group of centrally active drugs including amphetamine and phenmetrazine as well as some antidepressive substances, such as Imipramine.[7] Most of these substances including Imipramine evoked a rise in amount of ATP and a drop of ADP level. Our results are in good agreement with the findings of Lewis and Van Petten.

Prothiadene resembles Imipramine in its effect on the rat brain nucleotide level. Neither Prothiadene nor Imipramine, at a dose of 50 mg/kg body weight. had a

marked effect on the adenine nucleotide level 1 hr after administration. However, 3 hr after application, the same dose of Prothiadene caused an elevation of brain ATP level of up to 22·2 per cent and a significant drop in ADP and, contrary to the effect of Imipramine, in AMP levels, too. These changes are also reflected in the marked rise of the molar ratios of ATP to AMP and of ATP to ADP. It seems that these ratios, particularly the ratio ATP to AMP, reflect very sensitively the possible changes in the equilibrium state among individual phosphoric esters of adenosine and they can therefore be considered as critical indicators for possible interference in the metabolism of these nucleotides.

Marked elevation of UTP levels in rat brains was observed not only at 3 hr but also at 1 hr after administering Prothiadene and Imipramine. This significant rise of UTP levels was found even at a time when there had not yet occurred a marked change in the amounts of adenine nucleotides which are considered generally as the most sensitively reacting substances. This finding is at present an isolated observation and cannot therefore be easily explained. Possible causes are either a slowing in the utilization of UTP or an acceleration in its synthesis as is suggested by Lewis and Van Petten[7] for the changes of adenine nucleotides evoked by certain centrally active drugs.

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