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THE EFFECT OF ANTIDEPRESSIVE DRUGS AND SOME RELATED COMPOUNDS ON THE LEVELS OF ADENINE NUCLEOTIDES, INORGANIC PHOSPHATE AND PHOSPHOCREATINE IN THE RAT BRAIN

BY

J. J. LEWIS AND G. R. VAN PETTEN

From the Experimental Pharmacology Division, Institute of Physiology, University of Glasgow

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The effects upon levels of adenine nucleotides, phosphocreatine and inorganic phosphate of iproniazid, isoniazid, phenelzine, pheniprazine, tranylcypromine, harmine, imipramine, amitriptyline, orphenadrine, diphenhydramine and cocaine have been studied. With the exception of harmine and diphenhydramine, each of these compounds increased the brain level of adenosine triphosphate and, with the exception of imipramine and cocaine, the level of adenosine diphosphate decreased. Harmine had no effect on levels of adenine nucleotides and, in the case of diphenhydramine, the level of adenosine diphosphate increased and the level of adenosine triphosphate tended to decrease. There appears to be a relationship between the ability of the drugs to cause behavioural signs of central nervous stimulation and to produce an increase in the adenosine triphosphate/diphosphate ratio. This effect may be a factor in the action of antidepressive drugs.

Many attempts have been made to explain the mechanism of action of antidepressive drugs on the basis of their ability to inhibit monoamine oxidase, and thereby to increase the effective concentrations in the brain of its substrates, noradrenaline and 5-hydroxytryptamine, which have been postulated to act there as transmitters. Major difficulties arise, however, when an attempt is made to correlate the increase in the brain level of a particular amine produced by a drug with its antidepressive activity. Thus experimental evidence has been interpreted as indicating that the pharmacological effect is related to altered brain levels of noradrenaline (Brodie, Spector & Shore, 1959 ; Costa, Gessa, Hirsch, Kuntzman & Brodie, 1962), 5-hydroxytryptamine (Funderburk, Finger, Drakontides & Schneider, 1962) or normetadrenaline (Carlsson, Lindqvist & Magnusson, 1960 ; Carlsson, 1960). Moreover, the maximum levels of noradrenaline and 5-hydroxytryptamine found in the brain after the administration of inhibitors of monoamine oxidase may exceed those produced by complete inhibition of the enzyme. This suggests that other factors are involved in the increase in amine levels (Funderburk *et al.*, 1962 ; Dubnick, Leeson & Phillips, 1962). Indeed, the antidepressive drug imipramine increases the brain level of 5-hydroxytryptamine (Costa, Garattini & Valzelli, 1960),

although it does not significantly inhibit monoamine oxidase (Pulver, Exer & Herrmann, 1960).

On the other hand, the results of a previous investigation indicate that there is a relationship between the ability of amphetamine-like compounds to produce signs of central nervous stimulation in the rat and to increase the level of adenosine triphosphate and decrease the level of adenosine diphosphate in the brain (Lewis & Van Petten, 1962). The present investigation was undertaken, therefore, in an attempt to ascertain whether a similar relationship exists for a group of antidepressive drugs and some related compounds.

METHODS

The drugs used included iproniazid phosphate, isoniazid, phenelzine sulphate, (±)-pheniprazine hydrochloride, (±)-tranylcypromine sulphate, harmine hydrochloride, imipramine hydrochloride, amitriptyline hydrochloride, (±)-orphenadrine hydrochloride, diphenhydramine hydrochloride and (−)-cocaine hydrochloride; all doses refer to these salts. All drugs were dissolved in 0.9% saline, sterilized by filtration and administered by intraperitoneal injection using sterile tuberculin syringes and needles. With the exception of harmine and cocaine, in experimental series 5 (0.4 ml./100 g of body weight) and series 7 (1.0 ml./100 g) the drug solutions were such that 0.2 ml./100 g contained the required dose. An equivalent volume of sterile 0.9% saline served as the control.

Male Wistar rats, weighing from 75 to 95 g, were used. The order of treatment was randomized, using 3×3 latin square designs (Lewis & Van Petten, 1962). The rats were selected in groups of three of equal weight. One rat in each group received the control solution; the other two rats were treated with the drug solutions. The order of treatment and of all subsequent procedures were randomized by using three of the five possible 3×3 latin squares. The data obtained were evaluated by the analysis of variance appropriate to the design employed.

After injection of the drug or control solution, each rat was placed into a small cylindrical cage. The rats were not trained and had had no previous experience of the cages. The cages were kept in an air-conditioned room, which was electrically lit and had no natural illumination. Room temperature was constant at 20° C and noise was kept to a minimum.

Rats were killed, their brains frozen, and brain levels of adenine nucleotides, inorganic phosphate and phosphocreatine were determined as described by Lewis & Van Petten (1962).

RESULTS

After injection of the drug or control solution, the rats were placed in small cylindrical cages. The control animals soon settled and were comparatively inactive, and within 15 to 30 min were usually dozing or sleeping. Thereafter they would, from time to time, wake up, turn around in the cage, and then settle and go back to sleep. In contrast, the rats treated with iproniazid (20 and 40 mg/kg), phenelzine (5 mg/kg), pheniprazine (1 and 2 mg/kg), tranylcypromine (2 and 8 mg/kg) and cocaine (15 and 30 mg/kg) did not sleep as long as did the controls.

Isoniazid and iproniazid were found, by direct observation, to be equally effective in keeping the rats awake significantly longer than the controls. The rats treated with pheniprazine and tranylcypromine also exhibited moderate hypermotility. Although the signs of stimulation disappeared about 2 hr after treatment with cocaine, they were evident for at least 3 hr after injection of the other drugs. With pheniprazine and tranylcypromine the effect was still conspicuous after 6 hr but had

TABLE 1

IN VIVO EFFECTS OF MONOAMINE OXIDASE INHIBITORS AND RELATED COMPOUNDS ON THE ADENINE NUCLEOTIDE CONCENTRATIONS IN THE RAT BRAIN

All values are the means (±s.e.) of determinations on nine rats, made 3 hr after injection of the drug or control solution. Significance of differences from control: * $0.05>P>0.01$; † $0.01>P>0.001$; ‡ $0.001>P$. The experiments on cocaine (series 6) were done in conjunction with diphenhydramine (see Table 4), but for comparison the results have been tabulated separately. In this and subsequent tables, AMP=adenosine monophosphate; ADP=adenosine diphosphate; ATP=adenosine triphosphate

Series	Treatment	Dose (mg/kg)	Concentrations (μmole/g frozen tissue) AMP	ADP	ATP	AMP+ADP +ATP	Ratio ATP/ ADP
1	Control	0	0·65±0·05	0·72±0·05	2·06±0·08	3·43±0·13	2·95±0·22
	Iproniazid	14	0·65±0·06	0·53±0·05†	2·18±0·14	3·37±0·20	4·26±0·36‡
	Phenelzine	5	0·72±0·06	0·49±0·05†	2·47±0·13*	3·68±0·19	5·30±0·36‡
2	Control	0	0·82±0·03	0·76±0·03	2·49±0·03	4·07±0·06	3·31±0·15
	Iproniazid	20	0·86±0·04	0·65±0·03‡	2·84±0·07‡	4·33±0·12	4·41±0·18†
	Isoniazid	20	0·75±0·06	0·59±0·03‡	2·92±0·05‡	4·16±0·10	4·96±0·26†
3	Control	0	0·78±0·05	0·75±0·05	2·44±0·07	3·97±0·11	3·32±0·18
	Iproniazid	40	0·71±0·03	0·48±0·02‡	3·02±0·10‡	4·22±0·13*	6·32±0·21
	Isoniazid	40	0·78±0·03	0·46±0·03‡	2·90±0·10‡	4·10±0·13	6·55±0·49
4	Control	0	0·96±0·04	0·85±0·05	2·47±0·08	4·29±0·08	2·98±0·21
	Pheniprazine	1	0·82±0·05	0·69±0·04	3·04±0·07†	4·56±0·12	4·51±0·32†
	Tranylcypromine	2	0·82±0·04	0·75±0·04	2·83±0·09†	4·40±0·04	3·85±0·27†
5	Control	0	0·89±0·04	0·88±0·03	2·61±0·07	4·37±0·09	3·00±0·14
	Harmine	20	0·92±0·03	0·85±0·05	2·51±0·13	4·28±0·15	2·99±0·19
	Cocaine	20	0·94±0·06	0·91±0·05	2·29±0·07	4·14±0·13	2·59±0·15*
6	Control	0	0·68±0·03	0·68±0·03	2·64±0·09	3·97±0·08	3·95±0·25
	Cocaine	30	0·69±0·06	0·75±0·03	2·39±0·15	3·94±0·22	3·28±0·16

largely disappeared after 12 hr. In contrast, within about 5 min of injecting harmine a tremor developed, which continued intermittently for about 45 min. The animals treated with imipramine slept about the same length of time as did the controls but were much more responsive to environmental stimuli such as noises. Amitriptyline and orphenadrine also appeared to keep the rats awake, but diphenhydramine, which only lacks the *ortho*-methyl group of orphenadrine, appeared to make the rats sleep more than the controls.

Changes in brain levels of adenine nucleotides, inorganic phosphate and phosphocreatine

Monoamine-oxidase inhibitors and related compounds

The brain levels of adenine nucleotides determined 3 hr after treatment with the drugs are shown in Table 1. There was no significant change in the levels of inorganic phosphate or adenosine monophosphate and, except after treatment with cocaine or harmine (experimental series 5) which caused a significant decrease, there was no change in the level of phosphocreatine. An increase in the total content of adenine nucleotides occurred only after injection of 40 mg/kg of iproniazid (series 3). Although the adenosine triphosphate/diphosphate ratio was lower after 20 mg/kg of cocaine (series 5), the levels of adenosine di- and triphosphate did not change significantly either after this dose or after 30 mg/kg (series 6). Harmine

(20 mg/kg, series 5) did not change the ratio or the levels of adenosine di- and triphosphate.

After the other drugs there was, however, a rise in the adenosine triphosphate/diphosphate ratio, and the level of adenosine triphosphate increased significantly after phenelzine (5 mg/kg, series 1), iproniazid and isoniazid (20 and 40 mg/kg, series 2 and 3) and pheniprazine and tranylcypromine (1 and 2 mg/kg respectively, series 4). The decrease in the level of adenosine diphosphate was significant after each of these drugs except pheniprazine and tranylcypromine.

The effects 30 min after treatment with harmine, when the tremor was distinct, were studied, and the levels of adenine nucleotides are shown in Table 2. Neither 10 mg/kg, which produced mild tremor, nor 40 mg/kg, which produced considerable tremor, significantly changed the brain levels of the adenine nucleotides, phosphocreatine or inorganic phosphate (series 7). At 1.5 hr after the injection of cocaine (15 and 30 mg/kg), when signs of central nervous stimulation were apparent, both

TABLE 2

IN VIVO EFFECTS OF HARMINE AND COCAINE ON THE ADENINE NUCLEOTIDE CONCENTRATIONS IN THE RAT BRAIN

All values are the means (±s.e.) of determinations on nine rats. Significance of differences from control: †$0{\cdot}01 > P > 0{\cdot}001$. For harmine the brain concentrations were determined 0·5 hr after treatment; for cocaine, 1·5 hr

Series	Treatment	Dose (mg/kg)	Concentration (μmole/g frozen tissue) AMP	ADP	ATP	AMP+ADP +ATP	Ratio ATP/ ADP
7	Control	0	0·94±0·07	0·75±0·05	2·48±0·14	4·17±0·14	3·40±0·32
	Harmine	10	0·82±0·06	0·72±0·06	2·50±0·06	4·05±0·12	3·65±0·31
	Harmine	40	0·77±0·06	0·62±0·04	2·70±0·13	4·09±0·17	4·52±0·36
8	Control	0	0·89±0·06	0·74±0·02	2·25±0·03	3·89±0·08	3·03±0·09
	Cocaine	15	0·85±0·05	0·69±0·02	2·51±0·08†	4·04±0·11	3·66±0·13†
	Cocaine	30	0·83±0·03	0·68±0·02	2·54±0·06†	4·06±0·05	3·75±0·19†

the level of adenosine triphosphate and the adenosine triphosphate/diphosphate ratio were significantly increased (series 8).

An attempt was made to ascertain whether an increase in this ratio occurred at different times (0.5, 3, 6 and 12 hr) after treatment with pheniprazine and tranylcypromine, when behavioural signs of central nervous stimulation were observed. There was no change in the levels of inorganic phosphate, phosphocreatine or total adenine nucleotide after any of these time intervals. The level of adenosine monophosphate was decreased 3 hr after pheniprazine (Table 3). However, 0.5, 3 and 6 hr after treatment with each drug, the adenosine triphosphate/diphosphate ratio was significantly increased, although after 12 hr it was back to the control level. The rise in the level of adenosine triphosphate and the fall in that of adenosine diphosphate were only significant 3 and 6 hr after treatment. Since the animals were showing slight signs of central nervous stimulation 0.5 hr after treatment and considerable signs after 3 and 6 hr, there seems to be an approximate relationship between the central stimulant action of these two drugs and the increase in the adenosine triphosphate/diphosphate ratio.

TABLE 3

IN VIVO EFFECTS OF PHENIPRAZINE (2 MG/KG) AND TRANYLCYPROMINE (8 MG/KG ON THE ADENINE NUCLEOTIDE CONCENTRATIONS IN THE RAT BRAIN AT VARIOUS TIMES AFTER TREATMENT

All values are the means (±s.e.) of determinations on nine rats. Significance of differences from control after the same time interval: *0·05>*P*>0·01; †0·01>*P*>0·001; ‡0·001>*P*

Series	Treatment	Time after treatment (hr)	Concentration (μmole/g frozen tissue) AMP	ADP	ATP	AMP+ADP +ATP	Ratio ATP/ ADP
9	Control	0·5	0·82±0·06	0·76±0·05	2·41±0·06	3·92±0·12	3·28±0·24
	Pheniprazine	0·5	0·72±0·04	0·63±0·04	2·57±0·13	3·93±0·16	4·20±0·34*
	Tranyl-cypromine	0·5	0·77±0·05	0·63±0·06	2·48±0·11	3·88±0·15	4·15±0·37*
10	Control	3	0·77±0·05	0·80±0·03	2·59±0·06	4·19±0·06	3·28±0·18
	Pheniprazine	3	0·60±0·05†	0·62±0·05†	3·13±0·08‡	4·36±0·12	5·26±0·45†
	Tranyl-cypromine	3	0·74±0·05	0·68±0·03†	2·82±0·06‡	4·24±0·06	4·33±0·29†
11	Control	6	0·82±0·06	0·82±0·06	2·33±0·09	3·98±0·15	2·93±0·23
	Pheniprazine	6	0·66±0·07	0·64±0·07†	2·70±0·11*	3·96±0·19	4·30±1·17*
	Tranyl-cypromine	6	0·77±0·08	0·66±0·04†	2·68±0·15*	4·12±0·10	4·20±0·39*
12	Control	12	0·77±0·08	0·81±0·05	2·64±0·07	4·23±0·11	3·38±0·29
	Pheniprazine	12	0·81±0·03	0·82±0·03	2·56±0·11	4·19±0·13	3·14±0·13
	Tranyl-cypromine	12	0·66±0·02	0·72±0·05	2·80±0·07	4·18±0·07	4·06±0·32

Other antidepressive drugs and related compounds

The effects of imipramine, amitriptyline, orphenadrine and diphenhydramine on the brain levels of adenine nucleotides 3 hr after treatment are shown in Table 4. None of these drugs significantly changed the brain levels of inorganic phosphate, phosphocreatine or the total adenine nucleotide content.

While the increases in the level of adenosine triphosphate and the adenosine triphosphate/diphosphate ratio and the decreases in the levels of adenosine di- and monophosphate were all significant after 25 mg/kg of amitriptyline, the same dose of imipramine had no significant effects (series 13). After the larger dose (50 mg/kg) of imipramine (series 14), there was a significant increase in the level of adenosine triphosphate but neither the level of adenosine diphosphate nor the ratio of the two nucleotides was changed significantly. When this dose of imipramine was repeated (series 15) the rise in both the level of adenosine triphosphate and the nucleotide ratio were significant. After 15 mg/kg of orphenadrine (series 14), the level of adenosine triphosphate and the nucleotide ratio increased significantly and, after 30 mg/kg, these changes were accompanied by a significant decrease in the level of adenosine diphosphate. In contrast, 30 mg/kg of diphenhydramine tended to have the opposite effects; there was a significant increase in the level of adenosine diphosphate, while the level of adenosine triphosphate and the ratio of the two nucleotides were higher than for the control, though not significantly (series 6). The tendency of the chemically closely-related compounds, orphenadrine and diphenhydramine, to have opposite effects is of particular interest since orphenadrine stimulates the central nervous system while diphenhydramine causes sedation.

TABLE 4

IN VIVO EFFECTS OF IMIPRAMINE, AMITRIPTYLINE, ORPHENADRINE, AND DIPHENHYDRAMINE ON THE ADENINE NUCLEOTIDE CONCENTRATIONS IN THE RAT BRAIN

All values are the means (±s.e.) of determinations on nine rats, made 3 hr after injection of the drug or control solution. Significance of differences from control: *0·05>*P*>0·01; †0·01>*P*>0·001; ‡0·001>*P*. The experiments on diphenhydramine were done in conjunction with cocaine (see Table 1), but for convenience in comparing the effects of diphenhydramine with the closely related compound, orphenadrine, the results are tabulated separately

Series	Treatment	Dose (mg/kg)	Concentration (μmole/g frozen tissue) AMP	ADP	ATP	AMP+ADP +ATP	Ratio ATP/ ADP
13	Control	0	0·87±0·03	0·87±0·03	2·42±0·04	4·16±0·06	2·81±0·09
	Amitriptyline	25	0·70±0·07*	0·51±0·05‡	2·83±0·11*	4·04±0·20	6·29±0·95†
	Imipramine	25	0·86±0·03	0·83±0·07	2·58±0·09	4·27±0·10	3·32±0·33
14	Control	0	0·80±0·02	0·79±0·02	2·46±0·05	4·05±0·06	3·11±0·07
	Imipramine	50	0·74±0·03	0·77±0·05	2·59±0·06†	3·92±0·12	3·46±0·21
	Orphenadrine	15	0·75±0·03	0·69±0·03	2·74±0·07†	4·16±0·07	4·04±0·23*
15	Control	0	0·85±0·07	0·90±0·03	2·44±0·07	4·19±0·09	2·73±0·11
	Imipramine	50	0·81±0·04	0·83±0·04	2·66±0·07†	4·31±0·08	3·25±0·21‡
	Orphenadrine	30	0·79±0·03	0·76±0·04*	2·82±0·02†	4·36±0·06	3·82±0·22‡
6	Control	0	0·68±0·03	0·68±0·03	2·64±0·09	3·97±0·08	3·95±0·25
	Diphen-hydramine	30	0·72±0·06	0·86±0·03†	2·55±0·06	4·14±0·09	3·01±0·16

DISCUSSION

The differences between values of adenine nucleotides within a given experimental series may be due to variations in the brain levels of these compounds from animal to animal, or to slight variations in technique during the dissection and subsequent extraction or in the rate at which the brain is frozen within the cranial cavity. Variations due to the last are not unlikely because of the presence in brain of a highly active adenosine triphosphatase which, unless rapid freezing techniques are employed, will destroy the labile phosphate esters within a few seconds. It is also possible that failure to standardize completely the environment in which the animals were kept prior to freezing may have altered the control values to some extent, and is thereby partly responsible for the variations. Slight changes in the technique of injection and in putting the animals in their cages may also have contributed. To minimize these effects, all of the operations in a given series were carried out by the same worker. Within a single series, however, analysis of variance indicated that variations in technique did not significantly influence the results obtained.

The actual *in vivo* brain levels of adenine nucleotides and phosphocreatine are not known, and the values reported in the literature vary widely. The method of killing, the extraction process and the assay procedure may each influence the results (Weiner, 1961). For example, freezing the brain after decapitation is generally found to give lower levels of adenosine triphosphate and higher ones of adenosine mono- and diphosphate than is total immersion of the animal, and the use of an anaesthetic prior to decapitation, while it may give higher levels of adenosine triphosphate (Minard & Davis, 1962), increases the difficulties of interpreting the results. The results described in this paper were obtained with a method in which the brain

was rapidly frozen *in situ* by total immersion of the rat in liquid nitrogen in order to obtain minimum breakdown of the phosphate esters, and so to give values as near as possible to those likely to be present *in vivo*.

Compounds which produced behavioural signs of central nervous stimulation in the rat also in general decreased the level of adenosine diphosphate and increased the level of adenosine triphosphate in the rat brain. This last effect could be due either to decreased utilization or to increased resynthesis of adenosine triphosphate. During anaesthesia the brain level of adenosine triphosphate remains unchanged or increases (Lin, Cohen & Cohen, 1958 ; Gerlach, Döring & Fleckenstein, 1958 ; Minard & Davis, 1962) and the specific radioactivity of adenosine triphosphate following the intracisternal injection of radioactive phosphate is greater (Bain, 1957), despite the fact that there is a decreased oxygen uptake (Kety, 1948 ; Wechsler, Dripps & Kety, 1951 ; Gordan, 1956). These results suggest that in anaesthesia there is, as well as decreased physiological activity, a decreased utilization of adenosine triphosphate by the brain. Conversely, if emotional excitement is produced in an experimental animal, either by rotating it in a drum (LePage, 1946) or by teasing it with a straw after the administration of amphetamine (Shapot, 1957), the brain level of adenosine triphosphate falls. In this case the brain is probably using a greater amount of adenosine triphosphate to support its increased activity. The increase in the brain level of adenosine triphosphate during central nervous stimulation by drugs (when presumably the brain also requires more adenosine triphosphate for its functions) indicates, therefore, that there is probably a net increase in the resynthesis of adenosine triphosphate.

The results of this investigation show no relationship between the abilities of a drug to inhibit monamine oxidase and to increase the brain level of the adenosine triphosphate. Thus iproniazid and isoniazid were equally effective in increasing the level of adenosine triphosphate and the signs of central nervous stimulation, although isoniazid is a comparatively weak inhibitor of monoamine oxidase (Zeller, Barsky, Fouts, Kirchheimer & Van Orden, 1952). The potent short-acting monoamine oxidase inhibitor, harmine (Pletscher, Besendorf, Bächtold & Gey, 1959), produced neither typical signs of central nervous stimulation nor an increase in the brain level of adenosine triphosphate.

Similarly, there appears to be no relationship between the increase in the brain level of adenosine triphosphate and the increase in levels of noradrenaline and 5-hydroxytryptamine shown to occur after the administration of antidepressive drugs. For example, the brain levels of these amines are increased after the administration of iproniazid, phenelzine, pheniprazine, tranylcypromine and harmine (Prockop, Shore & Brodie, 1959 ; Pletscher *et al.*, 1959 ; Green & Erickson, 1960 ; Crout, Creveling & Udenfriend, 1961), but the level of adenosine triphosphate did not change after harmine. Moreover, after pheniprazine and tranylcypromine the amine levels rise to a maximum after 5 to 6 hr and remain considerably above normal for 48 hr or more (Prockop *et al.*, 1959 ; Green & Erickson, 1960 ; Crout *et al.*, 1961). In contrast, the level of adenosine triphosphate is high 3 and 6 hr after the administration of these two drugs, and is normal after 12 hr. Although adenosine triphosphate may be involved in the synthesis (Kirshner, 1959), binding (Hillarp,

1960) or metabolism of noradrenaline (Axelrod, Albers & Clemente, 1959), the present results do not indicate any simple relationship between the increased level of adenosine triphosphate and any one of these factors.

The relatively close agreement between the ability of the drugs used in this investigation to increase the brain level of adenosine triphosphate and to produce behavioural signs of central nervous stimulation in the rat does, however, indicate that the former effect may be an important factor in their action on behaviour. It seems probable that an increased level of adenosine triphosphate may influence the maintenance of ionic gradients in the neurones of the brain, since a large amount of the energy made available by adenosine triphosphate is probably used for this purpose (Dawson & Richter, 1950; Heald, 1960; McIlwain, 1962).

At the moment we cannot be certain whether the effects upon brain levels of adenine nucleotides precede or result from changes in neuronal activity. However, increased behavioural activity is unlikely to be associated with an increase in the brain level of adenosine triphosphate and a fall in that of adenosine diphosphate if changes in these levels were secondary to the behavioural effects. The converse seems more probable. It is possible that increased neuronal activity produced by a different mechanism could, for example by initially increasing the utilization of adenosine triphosphate, lead to a new dynamic equilibrium between the utilization and resynthesis of the compound (Heald, 1960). Under the appropriate circumstances, a higher adenosine triphosphate/diphosphate ratio might be detected when the neuronal processes are arrested by fixing the brain. Nevertheless, the present results indicate that a shift in the dynamic relationship between utilization and production of adenosine triphosphate may be an important factor in the action of antidepressive drugs.

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REFERENCES

AXELROD, J., ALBERS, W. & CLEMENTE, C. D. (1959). Distribution of catechol-O-methyl transferase in the nervous system and other tissues. *J. Neurochem.*, **5**, 68–72.

BAIN, J. A. (1957). Barbiturates and certain aspects of phosphorus metabolism in the central nervous system. In *Progress in Neurobiology*, vol. 2, pp. 139–157, ed. WAELSCH, H. New York: Hoeker.

BRODIE, B. B., SPECTOR, S. & SHORE, P. A. (1959). Interaction of drugs with norepinephrine in the brain. *Pharmacol. Rev.*, **11**, 548–564.

CARLSSON, A. (1960). In *Adrenergic Mechanisms*, pp. 558–559, ed. VANE, J. R., WOLSTENHOLME, G. E. W. & O'CONNOR, M. London: Churchill.

CARLSSON, A., LINDQVIST, M. & MAGNUSSON, T. (1960). On the biochemistry and possible functions of dopamine and noradrenaline in brain. In *Adrenergic Mechanisms*, pp. 432–445, ed. VANE, J. R., WOLSTENHOLME, G. E. W. & O'CONNOR, M. London: Churchill.

COSTA, E., GARATTINI, S. & VALZELLI, L. (1960). Interactions between reserpine, chlorpromazine and imipramine. *Experientia*, **16**, 461–463.

COSTA, E., GESSA, G. L., HIRSCH, C., KUNTZMAN, R. & BRODIE, B. B. (1962). On the current status of serotonin as a brain neurohormone and in the action of reserpine-like drugs. *Ann. N.Y. Acad. Sci.*, **96**, 118–133.

CROUT, J. R., CREVELING, C. R. & UDENFRIEND, S. (1961). Norepinephrine metabolism in rat brain and heart. *J. Pharmacol. exp. Ther.*, **132**, 269–277.

DAWSON, R. M. C. & RICHTER, D. (1950). Effect of stimulation on the phosphate esters of the brain. *Amer. J. Physiol.*, **160**, 203–211.

DUBNICK, B., LEESON, G. A. & PHILLIPS, G. E. (1962). An effect of monoamine oxidase inhibitors on brain serotonin of mice in addition to that resulting from inhibition of monoamine oxidase. *J. Neurochem.*, **9**, 299–306.

FUNDERBURK, W. H., FINGER, K. F., DRAKONTIDES, A. B. & SCHNEIDER, J. A. (1962). EEG and biochemical findings with MAO inhibitors. *Ann. N.Y. Acad. Sci.*, **96**, 289–302.

GERLACH, E., DÖRING, H. J. & FLECKENSTEIN, A. (1958). Papierchromatographische Studien über die Adenin- und Gaunin-Nucleotide sowie andere säurelösliche Phosphor-Verbindungen des Gehirns bei Narkose, Ischämie und in Abhangigkeit von der Technik der Gewebsentnahme. *Pflügers Arch. ges. Physiol.*, **266**, 266–291.

GORDAN, G. S. (1956). Influence of steroids on cerebral metabolism in man. *Recent Progr. Hormone Res.*, **12**, 153–174.

GREEN, H. & ERICKSON, R. W. (1960). Effect of trans-2-phenylcyclopropylamine upon norepinephrine concentration and monoamine oxidase activity of rat brain. *J. Pharmacol. exp. Ther.*, **129**, 237–242.

HEALD, P. J. (1960). *Phosphorus Metabolism of Brain*, pp. 133, 157–160. Oxford: Pergamon.

HILLARP, N.-A. (1960). Some problems concerning the storage of catechol amines in the adrenal medulla. In *Adrenergic Mechanisms*, pp. 481–486, ed. VANE, J. R., WOLSTENHOLME, G. E. W. & O'CONNOR, M. London: Churchill.

KETY, S. S. (1948). Quantitative determination of cerebral blood flow in man. *Meth. med. Res.*, **1**, 204–217.

KIRSHNER, N. (1959). Biosynthesis of adrenaline and noradrenaline. *Pharmacol. Rev.*, **11**, 350–357.

LEPAGE, G. A. (1946). Biological energy transformations during shock, as shown by tissue analyses. *Amer. J. Physiol.*, **146**, 267–281.

LEWIS, J. J. & VAN PETTEN, G. R. (1962). The effect of amphetamine and related compounds on the concentration of adenine nucleotides, inorganic phosphate and creatine phosphate in the rat brain. *J. Pharmacol. exp. Ther.*, **136**, 372–377.

LIN, S., COHEN, H. P. & COHEN, M. M. (1958). The adenosine polyphosphates, creatine phosphate and ascorbic acid content of rat brain. *Neurology (Minneap.)*, **8**, suppl. 1, 72–74.

MCILWAIN, H. (1962). Appraising enzymic actions of central depressants by examining cerebral tissues. In *Enzymes and Drug Action*, pp. 170–198, ed. MONGAR, J. L. & DE REUCK, A. V. S. London: Churchill.

MINARD, F. N. & DAVIS, R. V. (1962). Effect of chlorpromazine, ether, and phenobarbital on the active-phosphate level of rat brain: an improved extraction technique for acid-soluble phosphates. *Nature (Lond.)*, **193**, 277–278.

PLETSCHER, A., BESENDORF, H., BÄCHTOLD, H. P. & GEY, K. F. (1959). Über pharmakologische Beeinflussung des Zentralnervensystems durch kurzwirkende Monoaminoxydasehemmer aus der Gruppe der Harmala-alkaloide. *Helv. physiol. pharmacol. Acta*, **17**, 202–214.

PROCKOP, D. J., SHORE, P. A. & BRODIE, B. B. (1959). Anticonvulsant properties of monoamine oxidase inhibitors. *Ann. N.Y. Acad. Sci.*, **80**, 643–651.

PULVER, R., EXER, B. & HERRMANN, B. (1960). Einige Wirkungen des N-(γ-Dimethylamino-propyl)-iminodibenzyl-HCl und seiner Metabolite auf den Stoffwechsel von Neurohormonen. *Arzneimittel-Forsch.*, **10**, 530–533.

SHAPOT, V. S. (1957). Brain metabolism in relation to the functional state of the central nervous system. In *Metabolism of the Nervous System*, pp. 257–262, ed. RICHTER, D. London: Pergamon.

WECHSLER, R. L., DRIPPS, R. D. & KETY, S. S. (1951). Blood flow and oxygen consumption of the human brain during anaesthesia produced by thiopental. *Anesthesiology*, **12**, 308–314.

WEINER, N. (1961). The content of adenine nucleotides and creatine phosphate in brain of normal and anaesthetized rats; a critical study of some factors influencing their assay. *J. Neurochem.*, **7**, 241–250.

ZELLER, E. A., BARSKY, J., FOUTS, J. R., KIRCHHEIMER, W. F. & VAN ORDEN, L. S. (1952). Influence of isonicotinic acid hydrazide (INH) and 1-isonicotinyl-2-isopropyl hydrazide (IIH) on bacterial and mammalian enzymes. *Experientia*, **8**, 349–350.