Biochemical Pharmacology, 1965, Vol. 14. pp. 729–738. Pergamon Press Ltd., Printed in Great Britain.

THE EFFECTS OF DRUGS ON UPTAKE AND EXIT OF CEREBRAL AMINO ACIDS

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(*Received* 21 *September* 1964; *accepted* 20 *November* 1964)

Abstract—The uptake and exit of amino acids in mouse brain could be influenced by a number of drugs *in vivo* and *in vitro*. Most of the drugs caused decreased uptake or exit, but they did not all act through the same mechanism. In brain slices *in vitro* the uptake of L-lysine and of cycloleucine was inhibited by reserpine or phenobarbital; pentylenetetrazole (Metrazol) under the experimental conditions had no effect. Chlorpromazine at higher concentrations also inhibited uptake; at lower levels uptake was slightly enhanced. Cocaine prevented inhibition by protoveratrine but not by ouabain and chlorpromazine. *In vivo* lysine uptake was unaffected by pentylenetetrazole and pentobarbital, strongly inhibited when pentobarbital was combined with hypothermia, temporarily decreased by intraperitoneal reserpine, and increased by intracerebral reserpine. The effects of the drugs were similar on cerebral cycloleucine uptake; reserpine and chlorpromazine slightly decreased uptake, and pentobarbital with cooling strongly decreased uptake; mescaline and lysergic acid had no effect. *In vivo* cycloleucine exit from the brain was decreased by reserpine and chlorpromazine and by pentobarbital with cooling. Part, but not all, of the effect was due to hypothermia. The possible mechanisms of action of the drugs on cerebral amino acid passage are briefly discussed.

THE properties of the mechanisms responsible for the passage of amino acids into and out of the brain in the living animal have been investigated in a number of laboratories in recent years.[1–6] Also, cerebral uptake was studied *in vitro* in brain slices for examination of amino acid transport in closer detail.[7–12] The studies showed that the uptake of amino acids by the brain, both *in vivo* and *in vitro*, occurs through mediated transport, and indicated the existence of several carriers for the various classes of amino acids.[3, 11, 13–15]

In the work reported here it was found that a number of drugs affected the *in vivo* and *in vitro* uptake and exit of amino acids in the brain. The effects were different with the various drugs and were also dependent on their concentration. Our purpose was to investigate the properties of the brain-barrier system and to establish whether drugs could be useful for such investigation. The various drugs can be expected to affect different aspects of the barrier system through different mechanisms, and therefore drug effects may shed important light on some properties of transport systems and on mechanisms that influence transport. Our studies do not indicate whether or not the pharmacological action of a drug occurs through its effect on the passage or on the levels of amino acids.

EXPERIMENTAL PROCEDURE

Most of the procedures employed have been described in previous publications; therefore only brief outlines are given here.

Animals, materials. Young adult (25–30 g) Swiss mice were used. The amino acids were purchased from the California Corp. for Biochemical Research; chlorpromazine was obtained from the Smith, Kline & French Laboratories; reserpine from Ciba Pharmaceutical Co.

Brain slices. After decapitation, the brains were rapidly removed; cerebellum and pons-medulla were discarded, and the rest of the brain was sectioned in a McIlwain tissue slicer.[16] About 150 mg tissue was incubated in 2 ml of a Krebs-Ringer medium containing 2 mM ^{14}C-labeled amino acid at 37° and pH 7·4 for 70 min in an O_2 atmosphere.[12] At the end of the incubation the rapidly filtered tissue slices were frozen in dry ice, weighed, and extracted with 10 volumes of 3% perchloric acid (PCA). The label of a portion of the PCA extract was measured in a Packard liquid scintillation spectrometer. The counting mixture was 100 ml toluene, 60 ml ethyleneglycolmonomethyl ether, 750 mg 2,5-diphenyloxazole, and 5 mg 1,4-*bis*-2(5-phenyloxazolyl)-benzene;[17] 0·5 ml of tissue extract was added to 16 ml counting mixture. From the content of label in the medium before and after, and in the tissue after the incubation, the amino acid concentrations were calculated and expressed as micromoles per milliliter slice water at the end of the incubation.

Brain slices swelled 30–40%, depending on the medium, and contained 86 per cent water at the end of the incubation. The calculated amino acid concentrations were corrected for the 8–10 per cent medium that adhered to the slices and are presented as micromoles per milliliter slice water rather than per milliliter intracellular water (they are not corrected for inulin space). The inulin space of the slices after correction for adherent medium was 50 per cent at the end of the incubation, and it was not significantly affected by the drugs. The only drug affecting the size of intracellular space was chlorpromazine; which, at the highest concentration tried, increased swelling and extracellular space. This effect, however, could not account for the high degree of inhibition caused by this drug. If inulin space were taken as a measure of extracellular space (in equilibrium with the medium), a correction for inulin space would yield intracellular levels of amino acids which would be considerably higher than the level calculated for total slice water.

In determining the amino acids by counting radioactivity it is assumed that the amino acids are not metabolized significantly during the experimental periods and therefore the content of the label is a valid measure of the amino acid levels. The metabolic stability of the amino acids used in our system seems to be well established.[18–19] ATP was determined in another portion of the PCA extract by means of the Calbiochem-Boehringer enzyme kits and procedures. This kit consists of P-glycerate, P-glycerate kinase, DPNH, and glyceraldehyde P-dehydrogenase in a triethanolamine buffer.

In vivo. The animals were injected as described in the legends to the tables; they were decapitated at the end of the experiment, and whole brain and heparinized plasma were frozen in dry ice, weighed, and extracted three times with 5 volumes of 3% PCA. Labeled cycloleucine was measured in the scintillation counter as described before; unlabelled lysine was determined after the removal of PCA on Dowex-2 columns[20] by a modification[21] of a microbiological assay.[22] Usually the animals were kept at room

temperature. To counteract the lowering effects on body temperature of some of the drugs, where indicated in the tables the animals were placed in a 31° incubator; this treatment kept body temperature at normal levels. For further cooling the animals were placed in a 0° chamber (an International refrigerated centrifuge with the rotor removed) until their body temperatures reached 20°; their temperature was kept at 20–23° by keeping the cooling chamber at 5–10°.

RESULTS AND DISCUSSION

Effects of uptake in brain slices

Brain slices upon incubation in appropriate media accumulate amino acids against a concentration gradient: at the end of the incubation the amino acid level in the tissue is severalfold higher than in the medium.[7–11] Of the drugs tried in the present study, chlorpromazine had a diphasic effect on lysine and cycloleucine uptake *in vitro*; lower concentrations slightly increased accumulation, and higher concentrations strongly inhibited it (Table 1). Lower chlorpromazine levels had a different effect from the

TABLE 1. THE EFFECT OF CHLORPROMAZINE ON AMINO ACID UPTAKE BY BRAIN SLICES

Amino acid	Inhibitor	μmoles amino acid per ml water			
		In medium	In tissue	Tissue/medium	Change (%)
L-Lysine		1·60	4·56	2·85 ± 0·11	
	Chlorpromazine 10^{-2}M	1·85	2·15	1·16 ± 0·08	−91
	2×10^{-3}M	1·78	2·54	1·43 ± 0·04	−77
	10^{-4}M	1·66	4·93	2·97 ± 0·21	+6
	2×10^{-5}M	1·62	4·82	2·98 ± 0·12	+7
Cycloleucine*		1·31	9·40	7·18 ± 0·37	
	Chlorpromazine 10^{-2}M	1·88	1·94	1·03 ± 0·08	−99
	2×10^{-3}M	1·70	2·64	1·55 ± 0·11	−91
	10^{-4}M	1·19	9·57	8·04 ± 0·89	+14
	2×10^{-5}M	1·21	9·50	7·85 ± 0·70	+11

* 1-Aminocyclopentane-1-carboxylic acid.

Averages of four experiments ± S.D. are given. Brain slices were incubated in a Krebs-Ringer medium for 70 min at 37° in O_2. The initial amino acid concentration in the medium was 2 mM. Per cent inhibition of concentrative uptake (uptake above medium levels) was calculated according to the following formula: (T/M ratio control − inhibited)/(T/M ratio control − 1) × 100.

higher ones in a number of systems. Lower concentrations of chlorpromazine (and azacyclonol) increased the incorporation of inorganic P into phospholipids in slices from brain but not from other organs, and higher concentrations caused a decrease in the incorporation.[23] The leakage of enzymes from isolated liver lysosomes into the medium was inhibited by lower and increased by higher levels of chlorpormazine.[24] At low levels the drug had no significant effects on metabolism,[23, 25] and its effects on permeability may have been the result of its interaction with membranes; the decreased leakage of lysomal enzymes suggests that this interaction in some respects stabilized the membranes. At higher levels chlorpromazine inhibited oxygen uptake and phosphorylation,[23, 25–27] and therefore its effect in inhibiting amino acid uptake may have been due to the decrease of available energy in brain slices. The lower energy content in

the slice caused by chlorpromazine inhibited the incorporation of glycine into brain slice proteins.[28] Thus at lower levels the drug may have stabilized membranes, while at higher levels it affected metabolism and energy supply. It is also possible that at higher levels the drug still interacted with membranes but, instead of stabilization, its effect was disruption. Interaction of chlorpromazine with membranes has been observed in a number of systems.[29, 30] The surface activities of a number of tranquilizers were noted, and they correlated with clinical potencies;[31] at higher levels the drug caused lysis of membranes.[32] The increased leakage of amino acids,[33] resulting from the partial disruption of the membrane by the drug, may thus also explain the decreased uptake by the slices at higher drug levels.

TABLE 2. THE EFFECT OF RESERPINE AND PENTOBARBITAL ON AMINO ACID UPTAKE BY BRAIN SLICES

Amino acid	Inhibitor	μmoles amino acid per ml water			
		In medium	In tissue	Tissue/medium	Inhibition (%)
L-Lysine		1·60	4·56	2·85 ± 0·11	
	Reserpine 10^{-3}M	1·81	2·94	1·62 ± 0·19	66
	10^{-4}M	1·62	4·49	2·77 ± 0·24	4
	Pentobarbital 10^{-3}M	1·77	2·72	1·54 ± 0·12	71
	10^{-4}M	1·62	4·20	2·59 ± 0·06	14
	Pentylenetetrazole 10^{-3}M	1·62	4·46	2·75 ± 0·13	5
Cycloleucine		1·31	9·40	7·18 ± 0·37	
	Reserpine 10^{-3}M	1·76	4·08	2·32 ± 0·29	79
	10^{-4}M	1·30	9·30	7·15 ± 0·38	5
	Pentobarbital 10^{-3}M	1·73	3·94	2·28 ± 0·15	79
	10^{-4}M	1·30	8·67	6·67 ± 0·32	8
	Pentylenetetrazole 10^{-3}M	1·33	9·40	7·07 ± 0·54	2

Averages of four experiments ± S.D. are given. For experimental conditions see Table 1.

Of the other drugs tried *in vitro*, reserpine and pentobarbital at higher concentrations inhibited uptake, while pentylenetetrazole (Metrazol) at these levels had no significant effect (Table 2). (The estimation of the proper medium concentrations of reserpine is made difficult by the low degree of solubility of this drug. Only small effects were found when the solubilizers, as they are supplied with the drug for injections, were omitted). The explanation for the inhibitions may again be either the interference with the supply of available energy in the slices or the interaction with membrane constituents. Somewhat similarly to chlorpromazine, a disphasic effect of reserpine on catecholamine release was recently observed;[34] lower concentrations of the drug inhibited and higher concentrations increased catecholamine outflux from storage granules in adrenergic nerve. It is clear from the above results (Tables 1–2) and from others,[35, 36] that inhibition of uptake *in vitro* is caused by a large number of compounds, not all of which may act through the same mechanisms. Some differences in the probable action of drugs are shown in the effects of cocaine on drug-induced inhibition of uptake (Table 3). Protoveratrine, ethylenediamine tetraacetate (EDTA), ouabain and chlorpromazine each inhibited lysine uptake *in vitro*, but, as was shown with L-DOPA,[37] the addition of cocaine decreased the inhibition by protoveratrine and

at times by EDTA, but did not alter the inhibition by ouabain or chlorpromazine. Morphine did not influence inhibition by the drugs. The effect of increased K concentration was also different on ouabain-induced as compared with protoveratrine-induced inhibition of DOPA uptake.[38] These observations suggest that the action of ouabain and chlorpromazine was in some way different from that of protoveratrine

TABLE 3. THE EFFECT OF COCAINE ON THE INHIBITION OF LYSINE UPTAKE BY BRAIN SLICES

Added compound	μmoles lysine per ml water			Inhibition (%)
	In tissue	In medium	Tissue/medium	
	1·66	4·95	2·98 ± 0·09	
Pv* 100 μM	1·70	2·95	1·74 ± 0·08	63
Pv 20 μM	1·68	3·41	2·03 ± 0·15	48
Pv 100 μM + cocaine	1·69	4·43	2·62 ± 0·14	18
Pv 20 μM + cocaine	1·65	4·85	2·94 ± 0·23	2
Pv 100 μM + morphine	1·69	2·92	1·73 ± 0·12	63
Cocaine 1 mM	1·59	5·41	3·40 ± 0·31	0
Morphine 1 mM	1·70	4·45	2·62 ± 0·12	18
Ouabain 10 μM	1·74	2·93	1·68 ± 0·11	66
Ouabain 10 μM + cocaine	1·72	2·99	1·74 ± 0·15	63
EDTA 100 μM	1·70	4·39	2·58 ± 0·20	20
EDTA 100 μM + cocaine	1·68	4·84	2·88 ± 0·24	5
EDTA 100 μM + morphine	1·71	4·42	2·58 ± 0·21	20
CPZ 2 mM	1·78	2·22	1·25 ± 0·09	87
CPZ 2 mM + cocaine	1·78	2·26	1·27 ± 0·11	86

* Pv = protoveratrine, EDTA = ethylenediamine tetraacetate, CPZ = chlorpromazine. When EDTA was added, Ca and Mg were omitted from the medium. The concentration of cocaine or morphine when added was 1 mM. Brain slices were preincubated with the drugs in a Krebs-Ringer medium for 30 min, then ^{14}C-L-lysine was added to bring the medium level to 2 mM, and the slices were incubated 1 hr at 37° in an O_2 atmosphere. (The results were similar when the preincubation step was omitted.) The averages of eight experiments ± S.D. are given.

in regard to amino acid uptake. It is of interest that protoveratrine, EDTA, and ouabain caused a decrease of K content in brain slices and that the decrease by all three was prevented by cocaine.[39] The effect of drugs on α-aminoisobutyrate uptake was similar to their effect on lysine uptake. Aminoisobutyrate uptake was inhibited by protoveratrine, EDTA, ouabain, and chlorpromazine. Cocaine in this case also decreased the inhibition by protoveratrine, but not the inhibition by ouabain or chlorpromazine. Morphine, which by itself was slightly inhibitory, did not alter the effect of the other drugs (Table 4). A further indication that all drugs did not act through the same mechanism was shown by the content of ATP of the slices at the end of the incubation. It could be expected that drugs inhibiting oxidative metabolism, and therefore decreasing uptake through decreasing the available energy supply for transport, would also decrease the content of ATP in slices. In preliminary experiments to be reported later, ATP levels were lowered by most of the inhibitors of uptake of amino acids *in vitro*, but not by all, ouabain decreasing uptake but not ATP levels.[40]

The role played by ATP in *in vitro* transport is not clear. With a number of inhibitors the per cent inhibition of uptake and per cent decrease of ATP were similar. Since, if uptake was about 50 per cent inhibited, ATP levels were also about 50 per cent of control, a "transport quotient"(uptake per ATP content) was the same in control and inhibited

slices in a variety of conditions, although not necessarily in all.[41] The dependence of uptake on ATP levels would make ATP either the rate-limiting factor in uptake or the substance determining the final equilibrium. Since accumulation of amino acids by the slices against a diffusion gradient is an energy-requiring process, the content of

TABLE 4. THE EFFECT OF COCAINE ON THE INHIBITION OF AMINOISOBUTYRATE UPTAKE BY BRAIN SLICES

Added compound	μmoles aminoisobutyrate per ml/water			Inhibition (%)
	In tissue	In medium	Tissue/medium	
	1·01	13·1	13·0 ± 0·8	
Pv 100 μM	1·32	6·56	4·97 ± 0·29	67
Pv 20 μM	1·18	11·1	9·41 ± 0·82	30
Pv 100 μM + cocaine	1·21	10·9	9·01 ± 0·71	33
Pv 20 μM + cocaine	1·05	12·8	12·2 ± 0·9	7
Pv 100 μM + morphine	1·30	6·43	4·95 ± 0·43	67
Cocaine 1 mM	0·90	14·0	15·6 ± 1·0	0
Morphine 1 mM	1·13	11·9	10·5 ± 1·0	21
Ouabain 10 μM	1·64	4·53	2·76 ± 0·30	85
Ouabain 10 μM + cocaine	1·68	4·98	2·96 ± 0·24	84
EDTA 100 μM	1·25	8·86	7·09 ± 0·81	49
EDTA 100 μM + cocaine	1·20	9·37	7·81 ± 0·90	43
EDTA 100 μM + morphine	1·27	8·67	6·83 ± 0·67	51
CPZ 2 mM	1·79	2·31	1·29 ± 0·13	98
CPZ 2 mM + cocaine	1·77	2·25	1·27 ± 0·17	98

Experimental details are as in Table 3, except that after the preincubation α-aminoisobutyrate instead of lysine was added. The averages of eight experiments ± S.D. are given.

available energy in the slices will be an important determinant of the degree of accumulation. The question, however, whether ATP can be directly utilized for transport, cannot be answered at present. The parallel decrease in uptake and ATP content of brain slices on a number of occasions would be an indication of ATP supplying the energy for amino acid transport, but the exceptions are not easy to explain. There are instances of decreased uptake in spite of normal ATP levels in the slices, as with ouabain, mentioned above and elsewhere;[40, 41] in these cases it must be postulated that the interference was not with ATP production but with its utilization. Another explanation could be that the direct energy supply of amino acid transport was not ATP but another high-energy compound,[42] usually in equilbrium with ATP. The fact that adding ATP to the inhibited slices did not restore amino acid uptake[40] may have been due to the lack of penetration of ATP into the slice.

Effects on uptake in vivo

It was of interest to establish whether drugs can alter cerebral amino acid passage in the living animal. It is obvious that the effect of a drug *in vivo* may occur through mechanisms different from those in brain slices. For example, cerebral circulation may be affected, although it has to be emphasized that the passage of amino acids is too slow to be limited by cerebral blood flow. *In vivo*, pentylenetetrazole and pentobarbital in the dose employed had no effect, while pentobarbital in combination with lower body temperatures greatly decreased lysine uptake (Table 5). Pentobarbital, and more strongly pentobarbital with cooling of the animal, decreased the incorporation of amino acids into brain proteins *in vivo*;[43] here a number of processes that involve amino acids may have been inhibited.

TABLE 5. THE EFFECT OF PENTYLENETETRAZOLE AND PENTOBARBITAL ON CEREBRAL LYSINE UPTAKE *IN VIVO*

Time (min)	μmoles lysine per 100 g fresh tissue, increase above control							
	Lysine alone		Pentylenetetrazole		Pentobarbital		Pentobarbital + cooling	
	Plasma	Brain	Plasma	Brain	Plasma	Brain	Plasma	Brain
5	260 ± 40	11 ± 1	210 ± 30	13 ± 1	270 ± 40	15 ± 3	290 ± 20	3 ± 1
10	280 ± 30	24 ± 3	260 ± 30	12 ± 3	280 ± 20	22 ± 2	270 ± 30	5 ± 1
20	270 ± 20	20 ± 2	320 ± 40	25 ± 4	260 ± 30	20 ± 2	350 ± 30	8 ± 2
60	260 ± 30	31 ± 2	250 ± 40	30 ± 4	380 ± 60	35 ± 4	430 ± 50	20 ± 2

Control values (without lysine administration): plasma 38 ± 2, brain 28 ± 1; with pentylenetetrazole: plasma 37 ± 4, brain 27 ± 1; with pentobarbital: plasma 34 ± 3, brain 28 ± 1; with pentobarbital and cooling: plasma 38 ± 2, brain 28 ± 2, μmoles lysine/100 g fresh tissue; in each case 60 min after the administration of the drug.

Swiss mice were injected i.p. with drugs and 0·3-ml portions of a solution (30 mg lysine/ml) at the beginning of the experiment and at 10 and 30 min. Pentylenetetrazole: 1 min before the first lysine injection 0·07 ml of a solution (30 mg drug/ml) was injected. Pentobarbital (Nembutal sodium Abott): 0·04 ml of a solution (50 mg drug/ml) was injected 5 min before the experiment. Pentobarbital and cooling: for the 5- to 20-min experiments lysine was injected at the beginning and at 10 min only; for the 60-min experiments, at the beginning only.

Single animals were used per experiment; the averages of three experiments ± S.D. are given.

TABLE 6. THE EFFECT OF CHLORPROMAZINE AND RESERPINE ON CEREBRAL LYSINE UPTAKE *IN VIVO*

Time (min)	Tissue	μmoles lysine per 100 g fresh tissue, increase above control				
		No drug	Chlorpromazine		Reserpine	
			i.p.	i.c.	i.p.	i.c.
5	Plasma	260 ± 40	310 ± 30		330 ± 40	
	Brain	11 ± 1	5·5 ± 1·5		8 ± 4	
10	Plasma	280 ± 30	280 ± 50	330 ± 40	260 ± 40	
	Brain	24 ± 3	4·5 ± 1·9	8 ± 3	7 ± 2	
20	Plasma	270 ± 20	330 ± 30	300 ± 30	380 ± 20	280 ± 40
	Brain	20 ± 2	19 ± 4	19 ± 2	20 ± 4	31 ± 5
60	Plasma	260 ± 30	300 ± 40	410 ± 50	560 ± 60	460 ± 30
	Brain	31 ± 2	20 ± 3	37 ± 7	44 ± 6	56 ± 9
60*	Plasma	460 ± 70				
	Brain	41 ± 3				

In each case 0·3 ml of a L-lysine solution (30 mg/ml) was injected i.p. in the beginning of the experiment and at 10 and 30 min. In the second 60-min experiment with no drug 0·5-ml doses were injected.

Intraperitoneal (i.p.) drugs: chlorpromazine, 0·1 ml of a 15 mg/ml solution 2 hr before the experiment, (50 mg/kg); reserpine, 0·3 ml of a 0·75 mg/ml solution 19 and 2 hr before the experiment (15 mg/kg). Intracerebral drugs (i.c.): 2 mg pentobarbital in 40 μlitres injected i.p. 5 min before the drug, then chlorpromazine, 60 μg in 5 μlitres; reserpine, 25 μg in 10 μlitres 1 min before the experiment.

The averages of four experiments ± S.D. are given.

* No drug.

Chlorpromazine inhibited lysine uptake, especially in short-term experiments; the results were similar whether the drug was injected intraperitoneally or intracerebrally. Intraperitoneally injected reserpine also temporarily inhibited uptake but, if this drug was injected intracerebrally, uptake temporarily increased above control uptake values (Table 6). The diphasic effects of chlorpromazine and reserpine in being

TABLE 7. THE EFFECTS OF DRUGS ON CEREBRAL CYCLOLEUCINE U PTAKE

Time (min)	Tissue	μmoles cycloleucine per 100 g fresh tissue					
		No drug	Reserpine	Chlorpromazine	Pentobarbital + cooling	Mescaline	Lysergic acid
30	Plasma	440 ± 90	440 ± 80	410 ± 60	560 ± 90	370 ± 40	400 ± 50
	Brain	88 ± 9	53 ± 9	82 ± 9	26 ± 4	99 ± 10	100 ± 4
60	Plasma	360 ± 50	380 ± 70	390 ± 30	410 ± 50	380 ± 30	390 ± 30
	Brain	160 ± 18	85 ± 10	100 ± 15	49 ± 7	150 ± 6	170 ± 8
180	Plasma	310 ± 30	300 ± 60	310 ± 40	310 ± 60	280 ± 30	310 ± 40
	Brain	220 ± 16	210 ± 8	170 ± 20	100 ± 14	200 ± 12	230 ± 14

Cycloleucine (0·6 ml of a 20 mg/ml solution) was injected i.p. at the beginning of the experiment. Reserpine and chlorpromazine were injected i.p. as described in the legends to Table 6, pentobarbital as in Table 5. Mescaline and lysergic acid (LSD-25) were injected i.p. 30 min before the beginning of the experiment at 60 mg/kg and 300 μg/kg respectively.

The averages of three experiments ± S.D. are given.

TABLE 8. THE EFFECT OF DRUGS ON CYCLOLEUCINE EXIT FROM MOUSE BRAIN

Time (min)	μmoles cycloleucine per 100 g fresh brain						
	Ether alone	Chlorpromazine	Reserpine	Pentobarbital	Pentobarbital + chlorpromazine	Pentobarbital + reserpine	Pentobarbital + cooling
Animals kept at room temperature, 25°							Kept at 6°
5	120 ± 13	130 ± 14	130 ± 12	130 ± 16	120 ± 7	130 ± 12	120 ± 4
30	65 ± 7	110 ± 16	130 ± 15	130 ± 21	100 ± 5	130 ± 20	120 ± 6
90	36 ± 5	65 ± 10	76 ± 13	74 ± 12	130 ± 16	120 ± 9	130 ± 6
180							110 ± 10
Body temperature	37–38	31–32	32–33	26–28	24–27	26–28	20–23
Animals kept in an incubator, 31°							
5		120 ± 7	130 ± 13	120 ± 6	120 ± 11	120 ± 4	
30		99 ± 17	110 ± 13	83 ± 8	76 ± 9	110 ± 4	
90		56 ± 8	65 ± 10	32 ± 8	70 ± 12	58 ± 9	
Body temperature		36–37	37–38	36–37	36–37	34–35	

Swiss mice were injected with 1 μmole cycloleucine in 10 microliters saline i.c. under light ether anesthesia at the beginning of the experiment. The animals fully recovered in 3–4 min. The drugs were injected i.p. as follows: chlorpromazine, 0·45 mg in 30 μliters 2 hr before the experiment (15 mg/kg); reserpine, 0·45 mg in 30 μliters 17 hr before and 0·30 mg in 20 μliters 2 hr before the experiment; pentobarbital as in Table 7.

The averages of three experiments ± S.D. are given.

different at low and high concentration have already been discussed. The effects of these drugs on the *in vivo* uptake of nonmetabolizable amino acid, cycloleucine, were similar to those obtained with lysine. Reserpine and chlorpromazine inhibited cycloleucine uptake in experiments of short duration and had no effect in longer experiments. Of the other drugs tried, mescaline and lysergic acid had no significant effect on cycloleucine uptake, while pentobarbital in combination with lowered body temperatures was strongly inhibitory (Table 7). It was well established that under the experimental conditions employed in the present study a significant portion of the administered chlorpromazine or reserpine pentrated the brain.[44–46] The fact that the drugs influenced amino acid penetration when injected intracerebrally indicates that their effect is directly on the brain. Significant changes were observed in the levels of a number of compounds in the free amino acid pool of the brain upon the administration of these drugs, but very little, if any, effect on the physiological lysine levels of the brain was observed.[47–49]

Not only the uptake but also the exit of amino acids from the brain was affected by the drugs tried (Table 8). Efflux of cycloleucine in each case was lower in the drug-treated animals than in the controls. In the case of pentobarbital, cycloleucine exit was completely inhibited initially, and adding chlorpromazine or reserpine or further lowering the body temperature caused no significant decrease of cerebral level during the experimental periods. Lower body temperatures resulting from administration of the drugs seemed to be partly, but not completely, responsible for the observed inhibition of exit. Reserpine and chlorpromazine (but not pentobarbital) decreased exit even when body temperatures were kept near normal levels (lower half of Table 8). The inhibition of cerebral tryptamine uptake by chlorpromazine *in vivo* seemed mostly due to hypothermia caused by the drug.[50]

The results discussed in the present study show that the uptake and exit of amino acids in the brain can be influenced by a number of compounds with pharmacological action, both under *in vivo* conditions and in brain slices. The experiments do not prove that the pharmacological action is mediated by affecting transport processes, although altered permeability may be responsible for some drug action. It is most likely that all drugs do not affect amino acid transport through the same mechanisms. Under certain conditions some drugs may react with membrane constituents, while other drugs influence specific metabolic reactions or the content of available energy in the brain. It is also likely that drugs can affect the transport not only of the amino acids but of other metabolites as well.

Acknowledgements—This work was supported in part by Public Health Service Research Grants NB-03226 and NB-04360 from the National Institute of Neurological Diseases and Blindness. The valuable help of Miss C. Diamond is gratefully acknowledged.

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