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Neurochemical Correlates of Behavior: Levels of Amino Acids in Four Areas of the Brain of the Rat During Drug-Induced Behavioral Excitation[1]

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MCBRIDE, W. J., J. N. HINGTGEN AND M. H. APRISON. *Neurochemical correlates of behavior: levels of amino acids in four areas of the brain of the rat during drug-induced behavioral excitation.* PHARMAC. BIOCHEM. BEHAV. 4(1) 53–57, 1976. – The levels of GABA, aspartate, glutamate, glycine and alanine were determined in 4 specific brain areas (telencephalon, diencephalon-mesencephalon, cerebellum and pons-medulla oblongata) of rats killed during a period of drug-induced behavioral excitation. Behavioral excitation was obtained in adult, male Wistar rats working on a Sidman shock-avoidance schedule following administration of 2 mg/kg tetrabenazine (TBZ) 18 hr after iproniazid (50 mg/kg) pretreatment. When compared to trained animals (working on the avoidance schedule but receiving no drugs), the excited rats had increased levels of GABA in the telencephalon and diencephalon-mesencephalon, decreased levels of aspartate in all 4 brain areas, and a lower content of glycine in the pons-medulla region. The changes in the levels of aspartate in all areas of the brain, GABA in the diencephalon-mesencephalon, and glycine in the pons-medulla were significantly correlated ($p<0.01$) with the degree of excitation. It was observed that avoidance training alone produced increases in the levels of four amino acids: aspartate in telencephalon and cerebellum, GABA in cerebellum, and glycine and glutamate in the pons-medulla. The injection of iproniazid alone or iproniazid followed by TBZ into naive animals had little effect on the levels of the five amino acids. The data are discussed in terms of aspartate and GABA interacting as neurotransmitters with cholinergic and catecholaminergic and/or serotonergic neurons to produce the behavioral excitation.

Amino acids Shock-avoidance Behavioral excitation Tetrabenazine Aspartate GABA Glycine Glutamate Alanine Iproniazid

BEHAVIORAL excitation can be produced for finite lengths of time in rats working on shock-avoidance schedules by injecting tetrabenazine (TBZ) 18 hr after iproniazid pretreatment [13,25]. Aprison and coworkers [3, 5, 14, 15, 25] have provided data which strongly indicate that some cholinergic pathways may be involved with maintenance of this type of behavioral excitation. However, we have also suggested the possibility that multiple transmitter pathways may be involved in various types of behavioral changes [3, 4, 6, 7]. Thus, two facts led us to consider certain amino acids in this type of behavioral excitation. First, the data we have provided which suggest a role for glycine and GABA as inhibitory transmitters and glutamate and aspartate as excitatory transmitters [9]. Second, the studies of Berl and coworkers [10,23] which showed that reserpine and pargyline could have an effect on the putative amino acid neurotransmitters aspartate, glutamate and GABA. These reasons and the fact that TBZ has reserpine-like effects [19,21] and iproniazid is a hydrazine-type monoamine oxidase inhibitor, which might have a direct effect on amino acid metabolism [20], led us to examine the levels of amino acids in four areas of the brain of rats killed during the period of behavioral excitation following iproniazid-TBZ administration.

METHOD

Behavioral Procedures

Five groups of adult male, albino rats (Wistar strain) were used in these studies. The first group of 9 animals received daily training sessions on a Sidman shock-avoidance schedule (RS_{20}:SS_{10}; 1.6 mA intensity; 0.5 sec duration) in a standard lever pressing apparatus [25]. After stable behavior was obtained, rats were preinjected (subcutaneously) with 50 mg/kg iproniazid phosphate about 17 hr before the session and were subsequently injected with

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TABLE 1

EFFECTS OF IPRONIAZID AND IPRONIAZID PLUS TBZ ON THE LEVELS OF AMINO ACIDS IN VARIOUS REGIONS OF THE CNS OF THE NAIVE RAT

Treatment	Alanine	Glycine	GABA	Aspartate	Glutamate
	μmoles/g tissue wet weight				
Telencephalon					
Saline + Saline	0.558 ± 0.028	0.754 ± 0.046	1.51 ± 0.08	2.51 ± 0.07	12.2 ± 0.13
Iproniazid + Saline	0.577 ± 0.005	0.774 ± 0.073	1.38 ± 0.05	2.79 ± 0.04*	12.6 ± 0.22
Irponiazid + TBZ	0.592 ± 0.020	0.807 ± 0.013	1.52 ± 0.06	2.46 ± 0.07	12.4 ± 0.38
Diencephalon + Mesencephalon					
Saline + Saline	0.420 ± 0.011	1.07 ± 0.06	3.37 ± 0.30	3.05 ± 0.06	12.5 ± 0.51
Iproniazid + Saline	0.388 ± 0.006*	1.15 ± 0.08	3.33 ± 0.17	3.24 ± 0.15	12.6 ± 0.40
Iproniazid + TBZ	0.444 ± 0.010	1.32 ± 0.11	3.48 ± 0.19	3.30 ± 0.14	13.3 ± 0.32
Cerebellum					
Saline + Saline	0.433 ± 0.008	0.518 ± 0.018	0.898 ± 0.063	2.14 ± 0.08	12.4 ± 0.52
Iproniazid + Saline	0.417 ± 0.021	0.585 ± 0.044	0.806 ± 0.068	2.39 ± 0.07	13.0 ± 0.23
Iproniazid + TBZ	0.453 ± 0.022	0.582 ± 0.036	0.970 ± 0.081	2.20 ± 0.04	13.2 ± 0.30
Pons-Medulla					
Saline + Saline	0.424 ± 0.028	3.83 ± 0.14	1.18 ± 0.07	2.70 ± 0.15	8.14 ± 0.40
Iproniazid + Saline	0.377 ± 0.016	3.57 ± 0.10	1.02 ± 0.09	2.54 ± 0.07	7.74 ± 0.28
Iproniazid + TBZ	0.421 ± 0.026	3.90 ± 0.09	1.15 ± 0.08	2.57 ± 0.08	8.48 ± 0.37

Data represent the means ± SEM from 4 individual naive animals within each of the subgroups. Statistical significance was determined with the student's *t*-test. Significance of difference between control and drug-treated animals are as follows: $*p<0.05$

TBZ (2 mg/kg subcutaneously) 30–60 min after the start of the session (once stable performance was established). They were then killed during the period of behavioral excitation (response rates at least 1.5 times control rates) which followed the iproniazid-TBZ administration [3]. The second group included 6 animals which were injected with saline instead of iproniazid and TBZ, and were similarly trained and were killed during an avoidance session. The last 3 groups included naive rats which were handled but not subjected to any training sessions and were killed following saline-saline injections (18 animals), iproniazid-saline injections (4 animals) or iproniazid-TBZ injections (4 animals) according to the same time sequence used in the first group.

Biochemical Procedures

All animals were killed by decapitation; the telencephalon, diencephalon plus mesencephalon, cerebellum and pons plus medulla oblongata were quickly removed, wrapped in aluminum foil and frozen between blocks of dry-ice. Samples were usually processed within 48 hr of killing. Tissue levels of alanine, glycine, GABA, aspartate and glutamate were determined with a GLC procedure [8,24].

RESULTS

The injection of iproniazid alone or iproniazid followed by TBZ into naive rats had very little effect on the levels of alanine, glycine, GABA, aspartate or glutamate in the CNS of the rat (Table 1). No apparent effects were observed on the gross behavior of the naive rats after injection of the drugs. However, when iproniazid and TBZ were given to animals trained to work on a Sidman shock-avoidance schedule, the rate of responding of these animals increased (mean response rate = 3.2 times control rates at time of sacrifice). At the time when these animals were killed (mean sacrifice time = 18 min following TBZ injection) the levels of GABA in the telencephalon and diencephalon-

TABLE 2

AMINO ACID LEVELS IN FOUR REGIONS OF THE BRAIN OF NAIVE RATS, SHOCK-AVOIDANCE TRAINED RATS, AND RATS DISPLAYING DRUG-INDUCED EXCITATION DURING SHOCK-AVOIDANCE BEHAVIOR

Treatment	Alanine	Glycine	GABA	Aspartate	Glutamate
			μmoles/g tissue wet weight		
Telencephalon					
Naive	0.532 ± 0.019	0.697 ± 0.038	1.34 ± 0.07	2.49 ± 0.10	12.9 ± 0.36
Trained	0.537 ± 0.011	0.838 ± 0.034	1.37 ± 0.07	2.98 ± 0.06*	14.8 ± 0.21*
Excited	0.539 ± 0.042	0.720 ± 0.058	1.93 ± 0.10*†	2.37 ± 0.21†	16.0 ± 1.10*
Diencephalon + Mesencephalon					
Naive	0.448 ± 0.012	1.09 ± 0.04	3.29 ± 0.22	3.03 ± 0.16	11.3 ± 0.28
Trained	0.403 ± 0.011	0.958 ± 0.052	2.11 ± 0.19*	3.31 ± 0.08	11.6 ± 0.36
Excited	0.394 ± 0.019*	1.14 ± 0.10‡	4.61 ± 0.25*†	2.33 ± 0.10*†	12.7 ± 0.69*
Cerebellum					
Naive	0.379 ± 0.017	0.511 ± 0.036	0.835 ± 0.016	2.07 ± 0.04	12.4 ± 0.34
Trained	0.441 ± 0.016	0.695 ± 0.040	0.896 ± 0.026*	2.27 ± 0.04*	14.0 ± 0.29*
Excited	0.374 ± 0.022	0.578 ± 0.058	1.04 ± 0.05*	1.76 ± 0.09*†	13.8 ± 0.67*
Pons + Medulla-Oblongata					
Naive	0.371 ± 0.022	3.26 ± 0.29	1.24 ± 0.11	2.54 ± 0.21	7.38 ± 0.53
Trained	0.341 ± 0.031	4.11 ± 0.27*	1.19 ± 0.06	2.97 ± 0.14	9.41 ± 0.74*
Excited	0.301 ± 0.009*	3.06 ± 0.08†	1.13 ± 0.07	1.88 ± 0.03*†	8.04 ± 0.19

The various brain areas were isolated from rats which were (a) not trained and given saline injections in place of iproniazid and TBZ (naive); (b) trained on the shock-avoidance schedule, given saline injections in place of iproniazid and TBZ, and killed during an avoidance session (trained); or (c) trained on the shock-avoidance schedule, injected with iproniazid and TBZ, and killed during the subsequent period of behavioral excitation (excited). For additional details see Experimental Procedures section. The animals used for the naive group were killed on the same days as the behavioral animals and are not the same animals used to obtain the data for the saline + saline group in Table 1.

Data represent the means ± SEM of 8–9, 5–6, and 13–14 determinations each for excited, trained, and naive groups, respectively (with one noted exception). Significance of difference at least at the level of $p<0.05$ is indicated by (*) with respect to the naive group and (†) with respect to the trained group.

‡N = 6

mesencephalon had increased while the levels of aspartate significantly decreased in all four brain areas with respect to the values found for the trained animals receiving no drugs (Table 2). The content of glycine was also lower in the pons-medulla region of the excited rats compared to the trained rats. The content of glutamate appeared to be elevated over control levels in both the excited and trained animals (Table 2). The levels of aspartate in the telencephalon and cerebellum, GABA in the cerebellum, and glycine and glutamate in the pons-medulla were higher in trained animals than in control rats.

A comparison of the change in the degree of excitation and the change in the levels of amino acids, using the levels obtained for the trained animals as the baseline control, showed that the changes in the levels of aspartate in all 4 CNS areas, GABA in the diencephalon-mesencephalon and glycine in the pons-medulla were significantly correlated with the degree of excitation (Table 3).

DISCUSSION

The most important finding in this study is that there appears to be a correlation with respect to degree of excitation (Table 3) and changes in the levels of (a) aspartate in

TABLE 3

CORRELATION BETWEEN CHANGES IN THE LEVELS OF AMINO ACIDS IN VARIOUS CNS AREAS OF THE RAT AND DEGREE OF DRUG-INDUCED BEHAVIORAL EXCITATION

Amino Acid	Average Change with Excitation* (%)	Correlation Coefficient (r)	p
	Telencephalon		
GABA	+ 41	0.401	NS
Aspartate	− 21	−0.747	<0.005
Glutamate	+ 8	−0.166	NS
	Diencephalon + Mesencephalon		
GABA	+118	0.709	<0.01
Aspartate	− 30	−0.821	<0.005
Glutamate	+ 9	0.034	NS
Alanine	− 2	0.175	NS
	Cerebellum		
GABA	+ 16	0.311	NS
Aspartate	− 22	−0.889	<0.005
Glutamate	− 1	−0.259	NS
	Pons-Medulla Oblongata		
Aspartate	− 37	−0.775	<0.005
Glycine	− 26	−0.670	<0.01
Alanine	− 12	−0.368	NS

Only those amino acids from the drug-induced excited animals which were statistically significant from the data obtained for either the trained or naive animals were tested for correlation with degree of excitation. The degree of excitation for any one animal was determined quantitatively by dividing the responses made during the 2 min period prior to death by the responses made during the 2 min prior to injection (see Aprison *et al.* [3], for additional information). The animals which were trained but not injected with drugs were used to obtain values for amino acids for a degree of excitation of 1.0. The range of the degree of drug-induced excitation was from 2.0 to 5.5.

*The average percent change with excitation was determined with respect to the values obtained for the trained animals which received no drugs.

the telencephalon, diencephalon-mesencephalon, cerebellum and pons-medulla; (b) GABA in the diencephalon-mesencephalon; and (c) glycine in the pons-medulla. These 3 amino acids have been suggested to be neurotransmitters [1, 2, 9, 11, 12, 22].

There have been several reports which suggest that there might be an interaction between catecholamine and amino acid metabolism in brain [10, 17, 18, 23, 27]. Tyce [27] reported that the combination of L-DOPA and a MAO inhibitor (β-phenylisopropylhydrazine) decreased the rate of ^{14}C incorporation from ^{14}C-glucose into glutamate, glutamine, aspartate and GABA. Nicklas *et al.* [18] reported that the combination of L-DOPA and a non-hydrazine MAO inhibitor (pargyline) decreased the levels of aspartate in 6 areas, increased the levels of glutamine in 5 areas, and increased the levels of GABA in 2 areas of the CNS of the rat. These latter investigators offer the hypothesis that the catecholamines in their role as neurotransmitters may also function as metabolic regulators of the amino acid transmitters. Our data would not only support the contention that there is an interaction between catecholamines and amino acids but it also suggests a third interaction between either or both of these two groups with acetylcholine [3,25].

In the present studies, the combination of iproniazid plus TBZ does not appear to have any marked effect on the levels of amino acids if given to naive rats (Table 1). However, the combination of these two drugs and the animals working in the shock-avoidance session appears to be required before significant changes are observed (Table 2). Using the same avoidance schedule, injection schedule, and dose of iproniazid plus TBZ, Aprison and coworkers have presented data which indicate that certain cholinergic pathways may be involved in the maintenance of the excitation observed [3, 5, 14, 15, 25]. However, Aprison *et al.* [3] also postulated, on the basis of their data, that certain catecholaminergic pathways may also be involved in the maintenance of this type of behavioral excitation. The combination of iproniazid plus TBZ may have its primary effect on the catecholaminergic and/or serotonergic neurons. Iproniazid causes an increased amount of monoamines to accumulate within neurons; subsequent administration of TBZ, which has reserpine-like effects [19,21], causes an increased release of monoamines from nerve endings. These released monoamines in turn act upon cholinergic neurons and cause an increased release of acetylcholine. Since atropine can block this excitation [14], it appears that the released acetylcholine is important for the maintenance of this type of behavior. The released acetylcholine in turn then may act upon neurons containing (and releasing as transmitter) aspartate and GABA. The interaction of these 4 or 5 transmitter systems are then required for the maintenance of this type of behavior. The training procedure somehow sensitizes certain acetylcholine, aspartate and GABA pathways and possibly certain catecholaminergic and/or serotonergic pathways; the behavioral excitability may then be triggered by the drug-induced release of catecholamines and/or serotonin. The concept of a functional interaction between dopaminergic and cholinergic neurons has received significant experimental support (for a review see [16]).

In all previous behavioral studies in our laboratory, we have not found any correlations between behavior and neurochemical changes in the pons-medulla region [7]. Therefore, at this time, we are not emphasizing the changes observed for glycine in the pons-medulla region until additional information can be collected.

Alternatively, the decreased levels of aspartate may be a result of its carbon atoms being channeled into the TCA cycle to help maintain a sufficient supply of carbon atoms for oxidation to help meet the increased energy demands of

the excited animals and/or for production of glutamate which is then utilized for the production of glutamine. In the present studies the levels of glutamine were not measured but the studies of Nicklas *et al.* [18], using injections of a MAO inhibitor in combination with L-DOPA, found that aspartate levels decreased while glutamine levels increased. Glutamine levels have been reported to increase as a result of increased cerebral activity [26,28]. Consequently, in the behaviorally excited animals aspartate may be utilized for the production of glutamate molecules which are then converted to glutamine. In the diencephalon-mesencephalon the production of glutamate molecules may be utilized for the production of GABA.

Interpretation of data in terms of a transmitter role for glycine, aspartate and glutamate is very difficult since these amino acids are most likely present in all cells in the CNS and may have several metabolic functions, in addition to a possible transmitter role. Consequently any interpretation of data for these amino acids must be worded cautiously and must take into consideration all the possible functions that may involve glycine, aspartate, and glutamate.

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