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Branched-Chain Amino Acids Increase the Seizure Threshold to Picrotoxin in Rats

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SKEIE, B., A. J. PETERSEN, T. MANNER, J. ASKANAZI, E. JELLUM AND P. A. STEEN. *Branched-chain amino acids increase the seizure threshold to picrotoxin in rats.* PHARMACOL BIOCHEM BEHAV **43**(3) 669–671, 1992. – During infusion of branched-chain amino acids (BCAAs) in humans, changes in ventilatory drive, appetite, and sleep have been reported. The mechanism by which BCAAs exert their effects on CNS remains unclear. Picrotoxin is a proconvulsant drug, acting as an antagonist on the GABA-benzodiazepine receptor complex. Twenty rats were randomized to receive either an IP injection with 4% BCAAs (300 mg/kg; 8 ml/kg) (n = 10) or placebo (saline 8 ml/kg) (n = 10). The mean latency time from injection to onset of seizures was recorded as an indication of the seizure threshold. Latency time was significantly longer for BCAAs than for placebo, 11.2 (±1.9) vs. 8.3 (±1.8) min. Thus, a BCAA injection increased the seizure threshold to picrotoxin ($p < 0.03$). This suggests that BCAA infusion may exert effects on the GABA-benzodiazepine receptor complex.

Branched-chain amino acids GABA-benzodiazepine receptor complex Proconvulsant drugs
Nutritional pharmacology

THE possible use of the stimulatory effects of supranormal amounts of certain nutritional components as therapy has attended growing attention and the field of nutritional pharmacology is evolving (4).

The branched-chain amino acids (BCAAs), valine, leucine, and isoleucine, have metabolic properties and effects that may be unique in several aspects: Their initial metabolism occurs mainly in the periphery, particularly in skeletal muscle, rather than in the liver. One or more of the BCAAs may exert specific regulatory effects on the rates of protein degradation and synthesis in skeletal muscle. Finally, BCAAs seem able to influence neurotransmitter levels in the brain with effects on behavior (13).

Few studies have been performed to evaluate the CNS effects of increased BCAA plasma levels. In previous studies in humans, we observed changes in the ventilatory drive, sleep pattern, and appetite/food intake during infusion with BCAAs (5,7,14,15). BCAA administration reduces the development of fatigue during prolonged exercise (2). Clinical studies employing intravenous BCAAs in hepatic encephalopathy (HE) indicate that patients in hepatic coma awake at least as quickly in response to administration of BCAAs and hypertonic dextrose solution as they do in response to conventional treatment and both regimes work much quicker than placebo (9).

The mechanisms responsible for these CNS effects remain unknown. The hypothesis has been put forward that an increased concentration of BCAAs in plasma will decrease the rate and synthesis of 5-hydroxytryptamine (5-HT) (from tryptophan) into the synapse, so that a change in brain functions results (3).

GABA is the major inhibitory neurotransmitter in the brain. The heterogeneous GABA receptor exists in most inhibitory synapses in the CNS and can be regulated by both benzodiazepines (BDZs) and barbiturates. High-affinity recognition sites for BDZs are part of the GABA receptor complex on the plasma membrane of neurons in the brain (8,11). Synthetic BDZ agonists promote GABAergic neurotransmission, and hence the hypnotic and anxiolytic effects of this class of drugs, by binding to these sites (10).

The proconvulsant drug picrotoxin acts as an antagonist on the GABA-BDZ receptor complex (12). To evaluate the interaction between BCAAs and this receptor complex, we studied the seizure threshold to picrotoxin in rats given

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TABLE 1

PLASMA CONCENTRATIONS (±SD) OF BCAAs

	V	L	I	BCAA	T	BCAA/T
NaCl	170 ± 24	123 ± 23	78 ± 13	367 ± 50	93 ± 12	3.9
BCAA	374 ± 75	240 ± 49	187 ± 45	818 ± 181	85 ± 15	9.5

V, valine; L, leucine; I, isoleucine. Total BCAA concentration (V + L + I) and tryptophan (T) in μmol/l, as well as the BCAA/T ratio 60 min after an IP injection of BCAA compared with a group receiving normal saline.

BCAAs or placebo prior to seizure induction with picrotoxin. We observed an increase in seizure threshold that may indicate BCAAs exert a direct or indirect effect on this receptor complex with possible clinical implications.

METHOD

Twenty male Wistar rats were weighed and randomized to receive either an IP injection with a 4% BCAA solution (BranchAmin^R, Travenol Lab., Deerfield, IL) (300 mg/kg; 8 ml/kg; body temperature) (n = 10) or placebo (saline; 8 ml/kg; body temperature) (n = 10). Each 100 ml 4% BranchAmin contains 1.24 g valine, 1.38 g leucine, and 1.38 g isoleucine. After 120 min, rats received an IP injection with picrotoxin (10 mg/kg) (Sigma Chemical Co., St. Louis, MO). Picrotoxin was diluted in hot, sterile water. Each animal was observed for the onset of convulsions and the latency time from injection of picrotoxin to onset of seizures was recorded. The observer did not know if the rat was pretreated with BCAAs. After onset of seizures, rats were given a lethal injection of thiopentone IP. Statistical analysis was made using Student's t-test. $p < 0.05$ was considered statistically significant.

In a pilot study, 12 rats were given an IP injection with 4% BCAAs (BranchAmin; 300 mg/kg; 8 ml/kg) (n = 6) or saline (8 ml/kg) (n = 6). After 60 min, blood samples were drawn and analyzed for the amino acid profile. The samples were peproteinized with sulphosalicylic acid prior to analysis in a Biotronic Amino Acid Analyser (Biotronic, Maintal, Germany) fitted with an ion-exchange chromatography column and postcolumn ninhydrin detector. The results are listed in Table 1.

RESULTS

The results of the seizure threshold study are listed in Table 2. There was a significant increase in the latency time to onset of seizures in the group pretreated with BCAAs.

DISCUSSION

The BCAAs have interesting physiological properties with stimulatory effects that may be pharmacologically valuable.

TABLE 2

MEAN LATENCY TIME IN min (±SD) FROM INJECTION OF PICROTOXIN TO ONSET OF SEIZURES

	Latency time in min.
BCAA	11.2 ± 1.9
Placebo	8.3 ± 1.8*

*$p < 0.03$.

It has been demonstrated that a BCAA infusion acts as a respiratory stimulant as the arterial CO_2 tension decreases with an increase in the ventilatory response to CO_2 (7,15). In chronic renal failure patients on maintenance hemodialysis with sleep disorders, we recorded polysomnographic and respiratory data during nights with an infusion of saline (placebo) or a 4% BCAA solution and observed that a BCAA infusion was associated with a normalization of REM sleep and a significant decrease in $ETCO_2$ during sleep (14). Our findings suggest an increased ventilation during sleep after nocturnal infusion of BCAAs as well as an improvement of the sleeping pattern. In one patient with severe sleep apnea, a BCAA infusion was associated with a decrease in total numbers of apneas and a marked reduction in the severity of oxygen desaturation. In a recent study in premature infants, a significant reduction in apneic spells as well as an improved lung compliance was observed during BCAA infusion (1).

We observed a stimulatory effect on appetite and food intake by giving BCAA-enriched amino acid solutions (5). The reduction in food intake seen when total parenteral nutrition with standard amino acid solutions are administered (6) did not occur or at least was less pronounced when the amino acid solution contained a high proportion of BCAAs.

The effects of BCAAs in hepatic encephalopathy and the effects on ventilation, appetite, sleep patterns, and fatigue suggest that stimulation of the CNS occurs. BCAAs may influence serotonergic neurotransmission. BCAAs are believed to act in competition with other large neutral amino acids for transport across the blood-brain barrier, thereby limiting the entry of amino acids as tryptophan (3). Amino acids, as well as being neurotransmitters in their own right, are also precursors for many of the neurotransmitters (e.g., the catecholamines, 5-HT, histamine, and the peptide neurotransmitters). The entry of the aromatic amino acids, including tryptophan, into the brain will be affected by the relative plasma concentrations of other large neutral amino acids, particularly the BCAAs. The pilot study showed that the BCAA dose given was sufficient to achieve changes in the BCAA plasma concentrations as well as the ratio of BCAAs to tryptophan, which competes with the BCAAs for the entry over the blood-brain barrier.

In the present study, we observed that BCAAs given to rats demonstrated an anticonvulsant effect against the proconvulsant agent picrotoxin. Picrotoxin is a much used experimental proconvulsive agent that binds to the GABA-BDZ receptor complex (12). Because the seizure sensitivity to picrotoxin was influenced by BCAAs, this indicates that BCAAs exert a central effect via the GABA-BDZ receptor complex. The increase in seizure threshold found in our study may also have therapeutic aspects, and it should be tested if BCAAs given in combination with common anticonvulsants will potentiate their effects.

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