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The Antinociceptive Effects of Branched-Chain Amino Acids: Evidence for Their Ability to Potentiate Morphine Analgesia

T. MANNER,* D. P. KATZ†[1] AND J. ASKANAZI†

*Turku University Hospital, Department of Anesthesiology, Kiinamyllynkatu 4-8, 20520 Turku, Finland
†Montefiore Medical Center and the Albert Einstein College of Medicine, Department of Anesthesiology, 111 East 210th Street, Bronx, NY 10467

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MANNER, T., D. P. KATZ AND J. ASKANAZI. *The antinociceptive effects of branched-chain amino acids: Evidence for their ability to potentiate morphine analgesia.* PHARMACOL BIOCHEM BEHAV 53(2) 449–454, 1996.—The effect of branched-chain amino acids (BCAA) on pain threshold was studied in rats. Nociception was induced by the hot-plate analgesia meter, a method measuring supraspinally organized pain responses. After a single intravenous injection of BCAA (320 mg/kg), the percent change in latency time to the pain response significantly increased by 19% in 60 min, and by 22% in 75 min ($p < 0.005$), as compared to an injection of an equal volume of a standard concentration of an amino acid solution or physiological saline. Subsequently, we studied the interaction of BCAA with opioid-type analgesia. In combination with intravenously injected morphine (3 mg/kg), BCAA significantly potentiated and prolonged the action of morphine using the hot-plate test. From 5 min after morphine injection, the latencies to a pain response were markedly higher with the combination of BCAA and morphine (+80% and +89% at 5 min after morphine injection, if BCAA was administered 45 or 60 min prior to morphine injection, respectively) when compared with the effect of morphine alone (+13% at 5 min; $p < 0.005$). BCAA demonstrated analgesic effects, which, in combination with morphine, potentiated and prolonged the antinociceptive action of morphine. BCAA may represent a new adjunct treatment modality for acute and chronic pain, and give us further insight into the mechanisms of pain control.

Branched-chain amino acids Pain Antinociception Hot-plate analgesia test

THE BRANCHED-CHAIN amino acids (BCAA; valine, leucine, isoleucine) are known to exert various specific effects on the central nervous system (CNS) (25). Previous investigations have demonstrated that infusion of BCAA increases ventilatory drive (29), improves sleep pattern in patients with sleep disorders (15,28), and enhances mood and appetite (4,11). A recent study demonstrated that intraperitoneally administered BCAA increased seizure threshold to picrotoxin (a proconvulsant drug acting as an antagonist at GABA receptors) in rats (26,27). In addition, BCAA have been widely used clinically to alleviate the CNS affectations (disturbances in consciousness, hepatic coma) associated with advanced liver failure (20). The mechanisms for the effects of BCAA on CNS are not known, although actions through serotoninergic or GABAergic systems have been suggested. Several neurotransmitters are synthesized from amino acid precursors, and, hence, the rate of synthesis of neurotransmitters can be influenced via alterations in the brain amino acid pool. We hypothesized that BCAA might modulate the pain response.

The aim of the present study was to investigate the effects of intravenously administered BCAA vs. a standard amino acid solution and physiological saline on rat pain threshold using a hot-plate analgesia meter. Additional studies were performed to assess the possible interactions of morphine and BCAA on the pain response. The hot plate analgesia meter was used to measure antinociceptive drug actions experimentally in fully awake, conscious animals.

METHOD

The study protocol was approved by the Institutional Animal Care and Use Committee of Montefiore Medical Center.

[1] To whom requests for reprints should be addressed.

The animals used in the experiments described below were male Sprague–Dawley rats weighing 300–350 g. The animals were housed in standard conditions with normal lighting and free access to food and water ad lib. In the hot-plate test employed in the experiments described below, a hot-plate analgesia meter (Model 39-D, Innovators in Instrumentation Inc., CA) was used to measure hot-plate latency, i.e., the time to exhibit a response to the heat, as a measure of pain response (13). The hot-plate analgesia meter consists of a thermoconducting surface with built-in highly sensitive thermoswitches encased in a box with an automatic timing device. The hot plate was heated to 55°C ± 0.2°C and the animal was placed on the center. The latency time of the animal to show a nociceptive (pain) response from when it was first placed on the hot-plate analgesia meter was recorded. The nociceptive (pain) response of the animal was identified by a) licking of a hind paw, b) rapid movement (jumping) of the rear paws, or c) jumping out of the enclosure. The animal was removed immediately after demonstrating a pain response. However, to prevent thermal injury, a cutoff point of 20 s was set during the testing period.

Before administration of any material to be tested, each animal was pretested on the hot plate two to three times, with at least a 10-min interval between tests. The response time results were averaged to give a baseline measurement. The material to be tested was injected intravenously into a tail vein, and the latency time of the pain response was measured on the hot-plate analgesia meter as described above. The test data is expressed as the percent change in the animal's response to the test material as compared to the animal's baseline measurement (% change in latency time).

TABLE 1

COMPOSITIONS OF 4% BCAA SOLUTION (BRANCHAMIN) AND 4% STANDARD AMINO ACID SOLUTION (MADE BY DILUTING 8.5% TRAVASOL WITH STERILE WATER) USED IN THIS STUDY

	4% Branchamin	4% Travasol
Essential amino acids (mg/100 ml):		
Leucine	1380	263
Isoleucine	1380	203
Valine	1240	195
Phenylalanine		263
Methionine		246
Lysine		246
Histidine		186
Threonine		178
Tryptophan		76
Nonessential amino acids (mg/100 ml):		
Alanine		0.88
Glycine		0.88
Arginine		440
Proline		178
Tyrosine		17
Serine		
Cysteine		
Total:		
Amino acids (g/100 ml)	4	4
BCAA (% of total)	100%	16%
g/100 ml		0.68
Essential amino acids (% of total)	100%	44%

Effect of BCAA vs. Controls

The antinociceptive effects of intravenously administered BCAA was compared to an injection of an equal volume of a standard amino acid solution or 0.9% saline. After baseline measurements, the rats received in a randomized, double-blind fashion, a slow IV injection (in 4 min) of 8 ml/kg of either 4% BCAA solution (BranchAmin, Baxter Laboratories, Deerfield, IL), a 4% standard amino acid solution made by diluting 8.5% Travasol (Baxter Laboratories) with sterile water, or an equivalent volume of 0.9% saline. The composition of the BCAA and standard amino acid solution are given in Table 1. Following the injections, the hot-plate test was repeated at 30, 45, 60, 75, or 90 min. Each rat was used only at two time points after treatment, with a minimum of 30 min between each interval. This was done to prevent any learning bias from affecting the animals' response to the painful stimuli. There was a minimum of eight animals studied per time point. After completing the experiments the animals were not used in any subsequent studies.

Effect of BCAA and Morphine

Three experimental groups with a minimum of five rats per time point per group were studied to assess whether there was an interaction between the analgesic effects of BCAA and morphine, as a representative opioid analgesic agent. All the animals were pretested to obtain baseline measurements.

The rats were subsequently injected intravenously according to one of the three following protocols. Two groups were injected with BCAA at different starting times in an attempt to obtain the peak analgesic effects of BCAA:

1. Group I rats [saline (45) + Mo] were the control group and were injected with physiological 0.9% normal saline. The volume that the animal received was 8 ml/kg of body weight, as a control. Forty-five minutes after the injection of 0.9% saline each rat was injected intravenously with 3 mg/kg of body weight of morphine.
2. Group II rats [BCAA (45) + Mo] were injected intravenously with 8 ml/kg of body weight of the 4% BCAA solution. Forty-five minutes after injection of the 4% BCAA solution each rat was intravenously injected with morphine (3 mg/kg of body weight).
3. Group III rats [BCAA (60) + Mo] were injected intravenously with 8 ml/kg of body weight of the 4% BCAA solution. Sixty minutes after injection of the 4% BCAA solution, each rat was intravenously injected with morphine (3 mg/kg of body weight).

After the injection of morphine, groups of rats were tested for latency time of the pain response on the hot-plate analgesia meter, in accordance with the procedure described above, at the time point intervals of 1, 2, 5, 10, 15, 20, 30, and 60 min after injection. Animals were tested only at two additional time points after treatment.

Statistical analysis was performed using the Systat software (Elysian, IN). A one-way analysis of variance model was used. Post-hoc comparisons between experimental groups were made using the Tukey test. A p-value of less than 0.05 was considered to be statistically significant.

RESULTS

The individual latency time at baseline ranged from 4 to 8 s, with a mean of 6.8 ± 0.7 s, which corresponds to previously reported results obtained with rats of same size, sex, and strain (33). There were no adverse effects associated with any of the injections, and the animals treated in both sets of experiments were all conscious during the hot-plate test. The latency time to the pain response decreased slightly after injections of both the standard amino acid solution and saline (Fig. 1). In general, the percent decreases in the response time varied from 7 ± 7% to −19 ± 6% with standard amino acid solution, and from −2 ± 4% to −13 ± 4% with saline control (mean ± SE). After injection of BCAA, however, the latency time increased significantly at 45 ($p < 0.05$), 60 ($p < 0.01$), and 75 ($p < 0.05$) min compared with both standard amino acid solution and saline. The maximum effect, a percent increase by 19–22% in the latency time, was measured 60–75 min after injection of BCAA, after which the effect diminished.

Figure 2 illustrates the results obtained by combining BCAA with morphine. Morphine alone (Group I) had a peak analgesic effect (+61–63%) at 1–2 min after treatment, which returned to baseline in 20–30 min. In contrast, both groups of rats preinjected with BCAA (Groups II and III) had significantly greater latency time at 5 ($p < 0.01$), 15 ($p < 0.05$), 20 ($p < 0.05$), and 30 ($p < 0.01$) min after injection of morphine when compared with control [saline (45) + Mo]. Both groups of rats pretreated with BCAA displayed more prolonged analgesic tendencies when compared to the group receiving morphine alone. In fact, the results underestimate the interaction of BCAA and morphine, because the investigators were forced to rescue the animals from the hot plate, because there was a tendency for the BCAA/morphine-treated groups to exceed the set cutoff limit. At all time points there was potentiation of analgesia in rats preinjected with BCAA 60 min prior to morphine, and a maximum effect (+117 ± 29%, mean ± SE) was observed in this group at 15 min after morphine injection. Throughout the experiment, the combination of BCAA and morphine produced a greater analgesic response as compared to morphine alone.

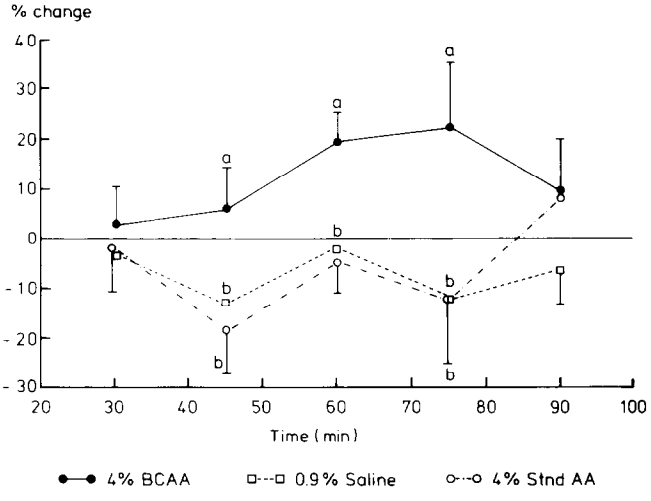

FIG. 1. The analgesic effects of a 4% BCAA solution, a 4% standard amino acid solution, and saline were compared. Rats were studied on a hot-plate analgesia meter, before and after treatment, and the percent change in latency time [(posttreatment response − baseline)/baseline × 100)] for the groups over time were compared. Each time point per group represents at a minimum of eight animals studied. Each animal was studied at only two time points after treatment. The data presented is the mean ± SE, and was analyzed by ANOVA, with post-hoc comparisons by Tukey test. Means with different letters at each time point are significantly different ($p < 0.01$). The data demonstrate that a 4% BCAA solution was significantly better than saline and a 4% standard amino acid solution at ameliorating the pain response.

DISCUSSION

The results of the present study indicate that BCAA plasma enrichment increased the pain threshold to thermal stimuli in rats. In addition, pretreatment with BCAA was found to potentiate and prolong the analgesic action of morphine. From a pharmacological perspective, it is noteworthy that there was a modest delay before the onset of the antinociceptive effect of BCAA. This finding suggests that BCAA may exert a nonspecific mode of action, and is not directly coupled to any specific receptor complex in the CNS. For example, BCAA may change the metabolic environment inside the blood–brain barrier (BBB). Amino acids, as well as being neurotransmitters in their own right, are also precursors for many of the neurotransmitters [e.g., catecholamines, serotonin (5-HT), histamine, and the peptide neurotransmitters]. In the CNS, there are enzymes available for prompt oxidation of the BCAA, thereby supplying energy substrates for the neuronal cells and/or precursors for the synthesis of neurotransmitters. In peripheral muscle tissue during metabolic stress, BCAA serves as a nitrogen donor for the formation of glutamine (25). Glutamine is the amide of the acidic amino acid glutamate, which is one of the excitatory amino acids and the precursor of GABA (gamma-aminobutyric acid), a major inhibitory neurotransmitter in the mammalian CNS. However, the role that BCAA may play in this aspect of brain metabolism has not been described.

A plausible mechanism where BCAA may directly affect neurotransmitter synthesis is by competing with other large neutral amino acids at the BBB for CNS entry. A good example would be the inhibition of the transportation of tryptophan (the precursor of 5-HT), which would result in decreased serotoninergic tone in the CNS (10). Serotonin is a neurotransmitter implicated in the central modulation of mood, ventilation, sleep, food intake, and pain (21). It is generally thought that increased serotoninergic tone decreases pain perception and descending serotoninergic pathways function at the spinal level by suppressing nociceptive input. However, complex interactions with other neurotransmitter systems confound the picture. Furthermore, it should be emphasized that an increase in the 5-HT synthesis rate does not necessarily parallel an enhanced 5-HT release into the synapse; excess 5-HT may either be accumulated and stored by synaptic vesicles, or metabolized by monoamine oxidase. Marsden and Curzon have demonstrated in unanesthetized rats that there is no increase in the rate of 5-HT release after tryptophan administration unless monoamine oxidase activity has been inhibited (18).

Human studies on the effects of tryptophan supplementation on the pain response have given mixed results. In patients with chronic pain, tryptophan-enriched diets have been found to increase pain tolerance (14). However, in the study by Ekblom et al. (9), in which tryptophan was given preoperatively, there was no effect on postoperative pain perception or on the

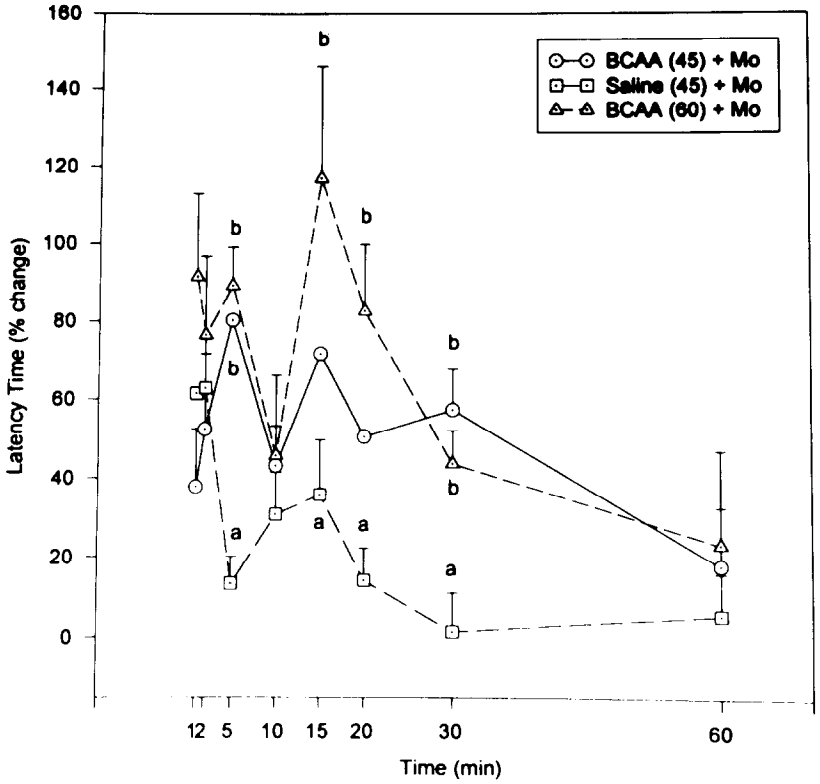

FIG. 2. The analgesic effects of saline and morphine [3 mg/kg; Saline (45) + Mo] and a combination of morphine (3 mg/kg) and BCAA administered either 45 min [BCAA (45) + Mo] or 60 min [BCAA (60) + Mo] prior to morphine injection were compared. Rats were studied on a hot-plate analgesia meter, before and after treatment, and the percent change in latency time [(posttreatment response − baseline)/baseline × 100)] for the groups over time were compared. Each time point per group represents at a minimum of five animals studied. Each animal was studied at only two time points after treatment. The data presented is the mean ± SE, and was analyzed by ANOVA, with post-hoc comparisons by Tukey test. Means with different letters at each time point are significantly different. Saline + Mo (Group I) had a peak analgesic effect (+61-63%) at 1-2 min after treatment, which returned to baseline in 20-30 min. In contrast, both groups of rats preinjected with BCAA (45) + Mo and BCAA (60) + Mo (Groups II and III) had significantly greater latency times at 5 ($p < 0.01$), 15 ($p < 0.05$), 20 ($p < 0.05$), and 30 ($p < 0.01$) min after injection of morphine when compared with control (saline + Mo). Both groups of rats pretreated with BCAA displayed more prolonged analgesic tendencies when compared to the group receiving morphine alone.

usage of additional analgesics. These contradictory findings may, in part, be explained by the different types of pain (acute vs. chronic) that were studied.

The potential mechanism of action for the antinociceptive action of BCAA may involve an interaction with the transmitter amino acids. The recent observation that BCAA prevented the onset of convulsions induced by picrotoxin (a noncompetitive GABA antagonist that blocks the chloride channel opening at the $GABA_A$ receptor site) in rats (26,27), suggesting that BCAA may influence, directly or indirectly, the GABAergic system. The role of the GABAergic system in the antinociceptive mechanisms is complex and involves multiple interactions with other pain-suppressing processes. GABAergic inhibition can be influenced by several mechanisms. Some agents stimulate presynaptical GABA release or decrease GABA metabolism and reuptake, while others directly activate the GABA receptor or enhance GABA binding to the receptor. Some can modify coupling between receptor activation, and others directly influence chloride channel opening (30). Both directly ($GABA_A$ and $GABA_B$ agonists) and indirectly (GABA uptake inhibitors and GABA-transaminase inhibitors) acting GABAergic agents have been shown to produce analgesia in a variety of animal nociceptive tests. Analgesia produced by $GABA_A$ agonists has been found to be insensitive to naloxone, bicuculline, picrotoxin, and haloperidol, but appears to be related to the central cholinergic and noradrenergic pathways (22). The $GABA_B$ agonist, baclofen, produces analgesia that is insensitive to naloxone, bicuculline, and picrotoxin, whereas catecholamines seem to mediate this action (31). GABA may also play a role in opiate analgesia. Acute administration of morphine alters the GABA content of discrete brain and spinal cord areas, which are important in processing of nociceptive

information, while chronic exposure to morphine modifies the binding of GABA to its receptor (23). Systemic administration of GABAergic agents enhances opiate analgesia, whereas intracerebroventricular administration or direct application of GABAergic compounds into the central pain-controlling nuclei reduces morphine analgesia (24). In animal studies, stimulation of $GABA_A$ receptors has been shown to suppress enkephalin release for several hours (16). At present, there is no direct evidence for an interaction of BCAA and the GABA receptor complex.

Recent studies on pain mechanisms have given growing attention to the role of the excitatory amino acids (EAA) and the NMDA (*N*-methyl-D-aspartate) receptor system in pain processing (1). Neurochemical studies have demonstrated that EAA, such as glutamate, coexists with other peptide transmitters in the substance P-containing dorsal horn fibers of the spinal cord (3). Upon stimulation of nociceptive afferents, EAA appear to be coreleased with substance P, while antagonists of the EAA have been shown to reduce mono- and polysynaptic excitation in the spinal cord (2,6,7). Behavioral studies confirm the observations obtained in electrophysiological studies that intrathecal injection of NMDA or EAA agonists produces hyperalgesia and pain behavior, whereas NMDA receptor antagonists have been found to be antinociceptive against the different modalities of experimental pain (5,32). Recent studies suggest that NMDA antagonists more potently attenuate the central facilitation of dorsal horn wide-dynamic-range neurons evoked by repetitive C-fiber stimulation (wind-up phenomenon), which explains their efficacy in tonic pain (3,8,17).

BCAA may interact with the NMDA receptor complex, because degradation of BCAA generally proceeds first through a transamination step yielding glutamate. Assuming that such a transformation in part mediates the analgesic properties of BCAA, it could act, directly or indirectly, to antagonize the function of the NMDA receptor complex. In previous electrophysiological and behavioral studies, it has been shown that NMDA receptor antagonists, such as AP-5 and ketamine, significantly inhibit the late, long-lasting tonic pain, but have only a small effect on the phasic response (12,19). If one accepts the hypothesis that BCAA antagonizes the NMDA receptor, the analgesic potency of BCAA would, therefore, be more dramatic in a tonic model of nociception, such as seen in postoperative or chronic pain.

In conclusion, this study demonstrates that an intravenous infusion of BCAA ameliorates the effects of painful thermal stimuli in rats. In addition, BCAA significantly potentiated and prolonged the antinociceptive effect of morphine. The study of the analgesic properties of BCAA and its ability to potentiate the action of morphine may give use new insights into the regulation of the pain response. Future research will address the mechanisms responsible for the antinociceptive actions of BCAA and the possible clinical relevance in the treatment of pain.

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