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The Effects of Branched Chain Amino Acid Infusion on Pain Perception and Plasma Concentrations of Monoamines

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KIRVELÄ, O. A., J. JAATINEN, H. SCHEININ AND J. KANTO. *The effects of branched chain amino acid infusion on pain perception and plasma concentrations of monoamines in healthy volunteers.* PHARMACOL BIOCHEM BEHAV **60**(1) 77–82, 1998.—Infusions of branched chain amino acids (BCAA) have been shown to have several CNS-mediated effects including antinociceptive action. We investigated the effects of BCAA infusion on pain perception, respiratory control, and plasma monoamine concentrations. Six healthy female volunteers were given in a double-blind, random, crossover design an 8-h infusion (1.75 ml/kg/h) of either (a) Ringers lactate, (b) conventional 4% amino acid solution, or (c) 4% BCAA solution with intervals of at least 48 h. Respiratory control was evaluated with continuous capnography. Pain perception was measured using dental dolorimetry for sharp pain, and pain transmitted by afferent C-fibers was evaluated with tourniquet test. Changes in vigilance were measured using critical flicker fusion technique. Evaluations were made for baseline, and after 2.5, 5, and 8 h. Plasma samples were collected at the same time points for amino acid and monoamine analysis. BCAA infusion resulted in significant increases of plasma concentrations of all BCAAs, with a simultaneous decrease in concentrations of aromatic amino acids. Of the measured monoamines and their metabolites dihydroxyphenylacetic acid (DOPAC) decreased, showing significant treatment effect for BCAA. Despite these changes no significant effect of BCAAs on respiratory control, vigilance, or pain perception was observed. In conclusion, despite significant changes in plasma concentrations of both amino acids and DOPAC, BCAA infusion did not show any clinically relevant antinociceptive effect. © 1998 Elsevier Science Inc.

Branched chain amino acids Pain Monoamines

BRANCHED chain amino acids (BCAA) valine, leucine, and isoleucine may have several specific pharmacological effects on central nervous system (CNS) function (16). Increased ventilatory drive (19), improved sleep pattern (7,18), and changes in mood and appetite (1,4) have been demonstrated. Based on recent animal experiments, also anticonvulsive and antinociceptive actions of BCAAs have been suggested (9,15). In earlier human studies with volunteers (1,4,7,18,19) the possible analgesic effect of BCAA infusion has not been assessed.

The present study was carried out to investigate possible antinociceptive effects of infusions of BCAA-enriched solutions in man. We examined the effects of an 8-h infusion of BCAA on pain perception, vigilance, respiratory control, and on the concentrations of monoamines and their metabolites in plasma.

METHOD

Protocol

Six healthy female volunteers (age 23 ± 1.9 years, mean ± SD) were studied on 3 separate days. None of the subjects had a history of alcohol or drug abuse or signs of mental disease or renal or hepatic dysfunction, and none had received any medication for at least 1 month prior to the study. All measurements were performed after an overnight fast. From the previous evening on, no stimulants (e.g., coffee) were allowed. The study protocol was approved by the Ethics Committee of Turku University Hospital. The subjects were adequately informed before entering the trial, and gave their written informed consent.

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Each subject received, in a random, double-blind, crossover design with intervals of at least 48 h, an 8-h peripheral intravenous infusion of: (a) Ringers lactate (Ringersteril®, Medipolar, Oulu, Finland), Group R; (b) conventional amino acid solution, diluted to a final concentration of 4% (Levamin Normo Plus®, Leiras-Kabi, Turku, Finland), group AA; (c) BCAA-solution, (Branchamin® 4%, Travenol Laboratories, Deerfield, IL), group BCAA (Table 1). Randomization was performed by the hospital pharmacy, and infusion bags were wrapped in foil and coded in the pharmacy. The dose for BCAAs and AA was 0.07 g/kg/h. In all groups the infusion volume was 1.75 ml/kg/h. The infusion rate was 100–150 ml/h, which corresponds to 4–6 g amino acids/h (0.443–0.664 g N/h) in groups AA and BCAA.

A small bore intravenous cannula (18 gauge) was inserted for plasma sampling. The first sample of plasma was collected immediately after the insertion of cannula to one 10.0 ml EDTA-plasma tube for monoamine analyses and one 10.0 ml Zn-heparin tube for amino acid analyses. Three other samples were collected at 2.5, 5, and 8 h. Blood for the chemical determinations was collected into chilled polypropylene tubes with K_2EDTA, which were promptly chilled on ice and centrifuged at 0–4°C. The plasma samples were stored at −70°C until analyses.

Measurements

Using a nasopharyngeal sampling tube, end-tidal CO_2 was measured during the whole study period with a high-performance capnograph (Ultima, Datex Co, Espoo, Finland).

Analgesic effects and the effects on vigilance were measured at the plasma sampling time points. The pain transmitted by afferent C-fibers was assessed by a slightly modified tourniquet test. Ischemic pain was induced by inflating a sphygmomanometer cuff to 300 mm Hg for 90 s; thereafter, the subject indicated the intensity of pain on a 10 cm visual ungraded analogue scale (VAS), where 0 means "no pain at all" and 10 means "intolerable pain." Sharp pain was measured with tooth pulp stimulation using dental dolorimetry (Bofors pulpa tester PT-1, AB Bofors, Nobel-Pharma, Sweden). The apparatus is based on the use of constant current pulses of 10-ms duration at a frequency of 5 Hz, the intensity of which can be gradually increased and read off accurately and instantly. The amperage can be increased automatically from 0 μA to 125 μA. Following careful drying of the tooth with an air stream and isolation with cellulose rolls, the electrode was placed on the upper incisor. The electrical current was then gradually increased from zero until the subject, who was unable to see the instrument panel, first felt pain or discomfort. At that point the subject herself switched off the current by pushing the button of the hand electrode. The reading was recorded in microamperes (μA).

Vigilance was measured with the critical flicker fusion frequency technique (CFF) (17). In this method a flickering red light-emitting diode, 3 mm of diameter, 1 meter distance from the subject, is employed. The threshold frequency is the point at which a flickering light gives rise to a subjective sensation of steady light. It was obtained by increasing the frequency twice until the subject reported fusion, and by decreasing the fre-

TABLE 1

THE COMPOSITIONS OF BRANCHED CHAIN AND CONVENTIONAL AMINO ACID SOLUTIONS (PER 100 ML)

Amino Acids g/100 ml	BCAA g/100 ml	Conventional AA g/ 100 ml
L-Valin	1.24	0.28
L-Leucin	1.38	0.35
L-Isoleucin	1.38	0.25
L-Threonin	—	0.19
L-Lycin	—	0.25
L-Methionin	—	0.12
L-Phenylalanin	—	0.24
L-Tyrosin	—	0.012
L-Histidin	—	0.15
L-Cystein	—	0.03
L-Aspartic acid	—	0.14
L-Alanin	—	0.45
L-Tryptophan	—	0.06
L-Arginin	—	0.38
Aminoacet. acid	—	0.15
L-Prolin	—	0.35
L-Serin	—	0.33
L-Glutamic acid	—	0.24
Amino acids total	4.0	4.0
Nitrogen total	0.443	0.6

TABLE 2

PLASMA CONCENTRATIONS (IN MICROMOLES/l) OF KEY AMINO ACIDS BEFORE (BASELINE) AND AFTER INFUSION (8 h) OF BRANCHED CHAIN AMINO ACIDS (BCAA), CONVENTIONAL AMINO ACID SOLUTION (AA), AND RINGERS LACTATE (RINGER) (MEAN ± SD)

	BCAA		AA		Ringer		
	Baseline	8 hours	Baseline	8 hours	Baseline	8 hours	Summary of ANOVA (treatment* time interaction)
Leucine	107 ± 15	920 ± 338 (760% increase)	116 ± 14	235 ± 42 (103% increase)	125 ± 19	130 ± 25	$F[2, 10] = 96.89, p < 0.001$
Isoleucine	57 ± 6	859 ± 349 (1407% increase)	54 ± 10	152 ± 38 (181% increase)	58 ± 12	58 ± 11	$F[2, 10] = 101.23, p < 0.001$
Valine	209 ± 30	1331 ± 273 (537% increase)	232 ± 33	434 ± 90 (57% increase)	232 ± 33	216 ± 29	$F[2, 10] = 88.72, p < 0.001$
Methionine	26 ± 3	6 ± 4	26 ± 1	50 ± 12	28 ± 4	21 ± 2	$F[2, 6] = 30.01, p < 0.001$
Phenylalanine	52 ± 6	24 ± 5	53 ± 5	100 ± 19	56 ± 5	50 ± 5	$F[2, 6] = 30.62, p < 0.001$
Tryptophan	46 ± 9	19 ± 8	39 ± 14	53 ± 11	30 ± 13	21 ± 4	Not applicable[1]
Tyrosine	60 ± 15	13 ± 12	58 ± 10	39 ± 7	68 ± 17	49 ± 7	$F[2, 4] = 12.81, p = 0.018$[2]

[1]Could not be analyzed due to missing values.
[2]Treatment effect.

quency twice until the flickering was perceived again. The mean value of four recordings was calculated for each time point.

Chemical Determinations

Noradrenaline, adrenaline, 3,4-dihydroxyphenylglycol (DHPG, an important metabolite of noradrenaline), and dihydroxyphenylacetic acid (DOPAC, an important metabolite of dopamine) in plasma were determined in a single run using high-performance liquid chromatography with coulometric detection (HPLC-EC) (14). The sensitivities for plasma concentration determinations using this method are DHPG; 0.175, noradrenaline; 0.08, adrenaline; 0.05 nmol/l. All samples from one experimental session were analyzed in one assay.

Amino Acid Analysis

Plasma was deproteinized by addition of an equal volume of lithium citrate loading buffer containing 50 g/l sulphosalisylic acid, then centrifuged. The analyses were carried out with the LKB 4151 ALPHA PLUS amino acid analyzer (Pharmacia LKB Biotechology, Uppsala, Sweden) according to the manufacturer's instructions. Only baseline and 8-h samples were analyzed.

Statistical Analysis

The statistical analysis was performed using analysis of covariance (ANCOVA) or variance (ANOVA, plasma amino acid levels only) for repeated measurements, with two within factors (treatment and time), computed with BMDP2V programs (MBDP Statistical Software Inc, Los Angeles, CA). The baseline value for each variable was used as a covariate in ANCOVA. When variances were unequal, log-transformation of the data was performed. If a significant treatment effect or treatment × time interaction was observed, the analysis was continued with separate ANCOVAs or ANOVAs for each pair of treatments. A p-value of less than 0.05 was considered statistically significant, and when pooled orthogonal components showed nonsphericity, Greenhouse-Geisser adjusted p-values were used. The results are presented as means ± SD.

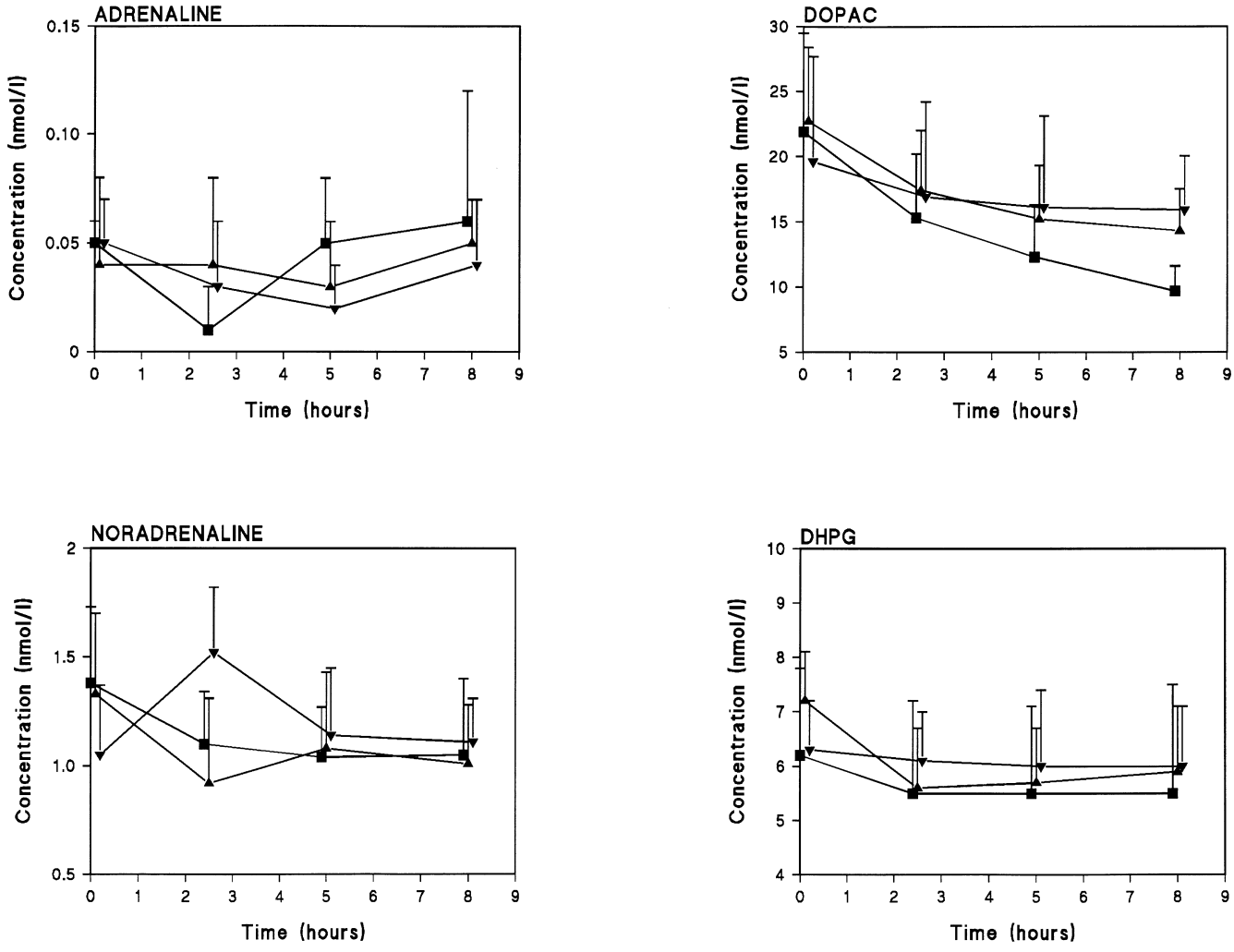

FIG. 1. Plasma concentrations of noradrenaline, adrenaline, DOPAC, and DHPG (nmol/l, means ± SD). Statistical analysis revealed significant differences between the groups in DOPAC [treatment effect: $F(2, 7) = 4.88$, $p = 0.047$], but in pairwise comparisons the BCAA group differed only from the placebo group, $F(1, 3) = 12.61$, $p = 0.038$. Differences in noradrenaline levels did not quite reach statistical significance (treatment × time interaction, $F(4, 16) = 2.76$, $p = 0.064$. ■ = BCAA, ▲ = conventional AA, ▼ = saline.

RESULTS

All infusions were well tolerated, and no adverse events were recorded. There were no differences in the baseline values between the treatments for any of the measured parameters. The overall statistical analyses showed significant differences in plasma concentrations of leucine, isoleucine, and valine (all $p < 0.001$) between the study groups. In the BCAA group, 5–14-fold increases from baseline were observed, but also in the AA group average increases of 90–180% in BCAA levels were evident (Table 2). In pairwise comparisons both treatments differed from the R-group (BCAA vs. R: $p < 0.001$ for all three BCAA's AA vs. R: $p = 0.003$–0.004) but also the differences between the two amino acid groups were statistically highly significant ($p < 0.001$ for all three).

There were statistically significant differences among the study groups also in some other key amino acids measured. During the BCAA infusion there were significant decreases in the plasma concentrations of the aromatic amino acids tyrosine ($p = 0.05$ vs. R) and phenylalanine ($p = 0.01$), and methionine ($p = 0.02$), while slight increases from baseline (except for tyrosine) were observed in the AA group (Table 2).

In the placebo group there was a slight and transient increase in plasma noradrenaline concentration at 2.5 h. The differences among treatments did not, however, quite reach statistical significance ($p = 0.06$). Plasma adrenaline concentrations remained at low levels in all three study groups throughout the infusions, and also the DHPG levels were comparable showing only time-related slight reductions (Fig. 1). Plasma DOPAC levels decreased in all three study groups, the changes being greatest in the BCAA group. Statistical analyses revealed a significant treatment effect for BCAA ($p = 0.05$), and in pairwise comparisons the BCAA group differed from the placebo ($p = 0.04$) but not from the conventional amino acid group ($p = 0.1$).

There were no significant differences among the treatments in tourniquet-induced pain ($p = 0.8$ for treatment × time interaction) nor in the dental pulp stimulation test ($p = 0.5$) (Fig. 2). No significant time effects were detected. Also, the degree of sedation as assessed by the CFF threshold test was relatively constant and similar ($p = 0.5$) in all three groups.

BCAA infusion did not significantly affect either respiratory rate ($p = 0.7$) or end-tidal CO_2 concentration ($p = 0.2$).

DISCUSSION

Branched chain amino acids, in addition to their nutritional role, have been suggested to exert several centrally mediated effects. These include ventilatory drive, sleep, mood, appetite, and gastric emptying. BCAA infusion has been shown to increase the ventilatory response to CO_2 after both an overnight fast (19) and a 1-week semistarvation (20). Kirvelä et al. demonstrated that nocturnal BCAA infusions have effects on respiratory control during sleep in healthy patients and in patients with chronic renal failure (7,18). Recently, the anticonvulsive and antinociceptive actions of BCAAs have been investigated in rats (9,15). The mechanisms for the effects of BCAAs on CNS are not known, although actions through serotoninergic or GABAergic systems have been suggested.

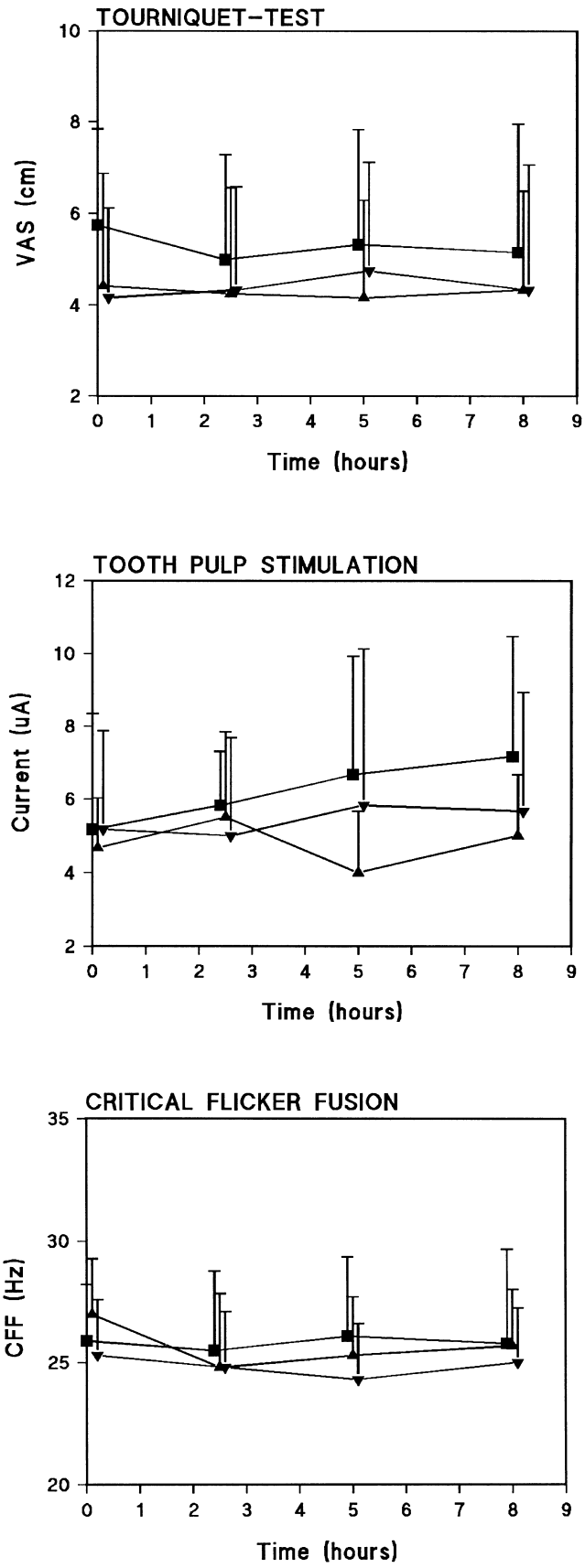

FIG. 2. Analgesic effects measured by tourniquet-test using VAS, tooth pulp stimulation; and vigilance measured by critical flicker fusion (means ± SD). No statistically significant differences between the groups. ■ = BCAA, ▲ = conventional AA, ▼ = saline.

From a pharmacological perspective, it is noteworthy that there has been a delay before the onset of the CNS-mediated effects of BCAA (1,4,7,9,15,18). This may suggest that BCAA exert a nonspecific mode of action, not directly coupled to any specific receptor complex in the CNS. 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. A plausible mechanism where BCAA may directly affect neurotransmitter synthesis is by competing with other neutral amino acids at the blood–brain barrier (BBB) for CNS entry. The rate of transport across the BBB for neutral amino acids is dependent upon the plasma amino acid concentration. In addition, competition between amino acids for a common transporter will occur so that the influx of an amino acid will be determined not only by its plasma concentrations and the K_m value but also by the plasma concentrations and K_m values of the other competing amino acids. The entry of the aromatic amino acids, including tryptophan, into the brain will thus be affected by the relative plasma concentrations of other large neutral amino acids, particularly BCAA. Inhibiting entry of tryptophan (2) may result in decreased production of serotonin (5-HT) (5) and its metabolite 5-hydroxyindolicaceticacid (3) and reduced serotonergic tone in the CNS. Serotonin is a neurotransmitter involved in the central regulation of mood, ventilation, sleep, food intake, and pain. Unfortunately, central activity or turnover of 5-HT could not be monitored in our subjects due to obvious ethical reasons.

If BCAAs alter the 5-HT system, there should be a mechanism mediating antinociception. This may occur through an interaction between the brain stem 5-HT and noradrenergic systems. A decrease in 5-HT inhibition of the noradrenergic activity augments noradrenaline release in the descending inhibitory tracts of the spinal cord with the ultimate result of potent suppression of pain conductance. Monoamines, noradrenaline, dopamine, and 5-HT act as neurotransmitters in human CNS. In the CNS the main dopamine metabolites are homovanillinic acid and DOPAC, and one of the main noradrenaline metabolites is DHPG. There is also evidence that BCAA may have activity on the GABA–benzodiazepine receptor complex (15). It should be borne in mind, though, that the role of the GABAergic system in the nociceptive mechanisms is very complex and involves multiple interactions with other pain-suppressing processes.

In our study, there was a slight reduction in plasma noradrenaline concentration in both amino acid groups, which probably is of minor importance. Plasma adrenaline concentrations remained low in all three study groups, and also the DHPG levels were comparable, showing only time-related slight reductions. Interestingly, BCAA infusion induced a more than 50% reduction in plasma DOPAC levels, suggesting a change in dopaminergic activity. Yet plasma levels of major dopamine metabolites (DOPAC or homovanillic acid) are relatively poor indicators of central dopaminergic activity.

An electrical pulp tester was used to determine the sharp pain threshold. Because the only sensation experienced by human subjects in response to activation of intradental nerves is slight discomfort or pain, electrical stimulation of tooth crown has been widely used as a model of experimental pain (6,10,11,12,21). Ischemic pain induced by a sphygmomanometer cuff is a simple and well-documented method of determining subjective pain intensity (6,13,17). In the present study, the negative results by using both of these methods give no indication that BCAAs could act as clinically usable analgesics.

In previous studies the BCAA infusion rate of 4.0 g/h has had effects on respiratory control and sleep pattern (7,18,19). In the present study, however, no effects on respiratory control or vigilance were observed. In the earlier studies we used male volunteers in contrast to females in this study, but otherwise the setting was similar. Although there are gender-related differences in pain sensitivities and thresholds (8), the lack of any measurable effect in this study speaks against a clinically significant antinociceptive effect.

The BCAA infusion in our study significantly increased the plasma concentrations of BCAAs and caused a significant decrease in plasma concentrations of aromatic amino acids (tyrosine, phenylalanine), and methionine. These changes are similar to those observed in earlier human studies were CNS effects of BCAAs have been demonstrated (4,18,19) and should, thus, be sufficient in generating CNS effects. Those effects have become evident within 4 h (4,7,18,19) and, accordingly, 8 h of BCAA infusion should have been sufficient for these effects to evolve. Increasing the dose of BCAA further is not clinically feasible due to the poor solubility of BCAAs and, thus, to the required infusion volume.

The minimum washout period in our study was 48 h. This should be sufficient, as the clearance of infused amino acids is very fast, leading to normalization of plasma amino acid profiles within a few hours. BCAAs are very fast, taken up by the liver and skeletal muscle and, accordingly, their concentration in plasma rapidly decreases to preinfusion values. In addition, there were no differences in baseline values between the treatments.

In conclusion, the possible antinociceptive function of BCAAs given by peripheral intravenous infusion could not be verified in the present study. Studies assessing the potential analgesic efficacy or analgesic potentiation of continuous BCAA infusions in clinical settings are needed before suggestions on their value in patient care can be made.

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