Effect of chain length of short-chain fatty acids on their effect on intracranial pressure in rabbits

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SUMMARY The short-chain fatty acids propionic, butyric, valeric, isovaleric and octanoic produced elevations in intracranial pressure during intravenous infusion in rabbits. Serum concentrations of these compounds are elevated in patients with Reye's syndrome, and may contribute to the intracranial pressure elevations found in these patients.

Reye's syndrome[1] is a severe metabolic encephalopathy occurring primarily in children. Mortality from this disease is high and it is one of the 10 most common causes of virus-related deaths in childhood.[2] Although it typically occurs following a virus infection, the aetiology of Reye's syndrome is unknown. Clinical features of the disease include vomiting, coma, hyperventilation, and seizures.[3] There is biochemical evidence of liver dysfunction, and the characteristic pathological change is diffuse hepatic microvesicular fatty infiltration. Increased intracranial pressure is a frequent complication and may be difficult to control.[4]

Elevations in serum concentrations of the short-chain fatty acids propionic, butyric, valeric, isovaleric,[5] and octanoic[6] are present in patients with Reye's syndrome, and serum concentrations appear to correlate with the clinical symptoms.[7] Infusion of sodium octanoate into rabbits produces clinical, biochemical and pathological changes similar to those found in Reye's syndrome,[8] and thus appears to be an experimental model for the disease. To test the effects of short chain fatty acids on intracranial pressure, the following study was performed.

Materials and methods

Twenty male 2 kg New Zealand albino rabbits were anaesthetised with ketamine 50 mg/kg and pentobarbital

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30 mg/kg. In each animal, the femoral artery was catheterised for blood pressure recording and the ear vein cannulated for infusion of test substances. A tracheotomy was performed and the animals mechanically ventilated with a Harvard volume respirator. An incision was made over the occiput and a 20 gauge catheter inserted into the cisternal space for intracranial pressure measurements. Needle electrodes were placed in the scalp over anterior and posterior head regions for electroencephalographic (EEG) recordings. Animals were paralysed with pancuronium bromide 1 mg/hr and allowed to awaken from the anaesthetic as determined by normalisation of blood pressure and a return of the EEG to an awake pattern. Blood pressure, intracranial pressure, EEG and temperature were recorded continuously throughout the experiment on a Beckman 611 8-channel polygraph. Arterial blood gas determinations were performed during the control period and hourly throughout the experimental period. pH, pCO_2, and pO_2 were maintained in the physiologic range by adjusting respiratory rate and volume on the mechanical ventilator.

Sodium propionate, butyrate, valerate, isovalerate and octanoate were infused intravenously in separate experiments as an 0·2 molar solution pH 7·4 at a rate of 0·13 ml/min continuously for 6 hours or until the animal died. All solutions were water soluble and free of precipitate. In some rabbits isovalerate was infused at an 0·3 molar concentration. Concentrations of the short chain fatty acids used were based on previous experiments in which octanoate at the same concentration produced the above described clinical, biochemical and pathological abnormalities.[8] Serum propionate concentrations were measured by the method of Ando *et al*.[9] Serum octanoate concentrations were quantitated by a gas chromatographic method described previously.[8]

Results

Intracranial pressure rose during infusion of sodium propionate, butyrate, valerate and octanoate

(table 1). Isovalerate at the same concentration produced no increase in intracranial pressure, but increasing the concentration to 0·3 molar caused a rise (table 1).

Toxicity of short chain fatty acids, as measured by survival time following onset of infusion, varied directly with increasing chain length, with the shortest chain compound (propionate) resulting in the longest survival, while octanoate infusion led to the shortest survival (table 2). The rapidity of production of increased intracranial pressure varied with chain length as well, with octanoate producing the most rapid changes.

Table 1 *Intracranial pressure elevation during short chain fatty acid infusion*

Short chain fatty acid*	Intracranial pressure max (mmH_2O) $\bar{x} \pm SEM$	p
Propionate (3)†	77·5 ± 18·8	0·001
Butyrate (4)	49·4 ± 2·6	0·001
Valerate (4)	51·3 ± 7·0	0·001
(a) Isovalerate (3)	34·2 ± 11·7	NS
(b) Isovalerate (3)	44·2 ± 5·8	0·001
Octanoate (4)	91·8 ± 26·4	0·001
Control ICP $\bar{x} \pm SEM$ = 24·5 ± 1·8 mmH₂O		

*All infusions used 0·2M concentration of sodium salt except (b) isovalerate which was 0·3M.
†Numbers in brackets indicate n for each group.

Table 2 *Toxicity of short chain fatty acids*

Short chain fatty acid	T max intracranial pressure (hr)	T survival (hr)
Propionate	7	8
Butyrate	5	6·5
Valerate	4	5·5
Isovalerate	4	6
Octanoate	2	2·3

Arterial pH, pO₂, and pCO₂ were monitored throughout the experiment, and although there was a trend toward decreased pO₂, the changes were not statistically significant (fig). Octanoate concentrations were undetectable in control serum and reached a maximum of 500 μM/l during infusion of octanoate. Serum propionate concentrations rose from control values of 0·43 μM/l to a maximum of 27·15 μM/l during propionate infusion.

Discussion

Numerous metabolic derangements are present in patients with Reye's syndrome.[3] One of the most intriguing is the elevation in plasma concentrations of the short chain fatty acids. Serum concentrations of these correlate with the clinical symptoms.[7] Several other diseases which produce coma and
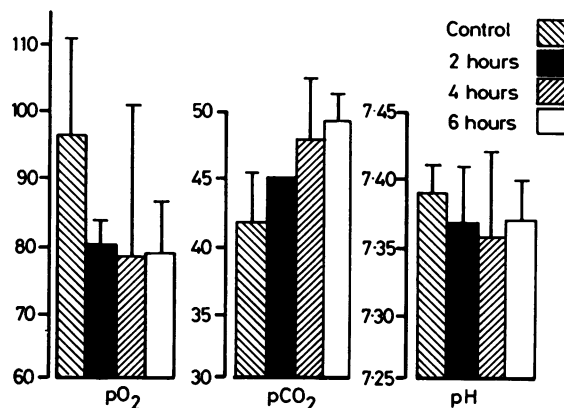

Figure *Arterial blood gas determinations during short chain fatty acid infusion ($\bar{x} \pm SEM$).*

hyperventilation share the common feature of short-chain fatty acidaemia. These diseases include Jamaican vomiting sickness,[10] Udorn encephalopathy,[11] hepatic encephalopathy,[12] and isovaleric acidemia.[13] The fact that several diseases with common clinical features share the same metabolic abnormality suggests a possible role of short chain fatty acids in production of the encephalopathy.

If the short chain fatty acid sodium octanoate is injected into rabbits, it results in coma, hyperventilation, seizures and EEG abnormalities.[8] Secondary metabolic abnormalities caused by such an injection include hyperammonaemia, lactic acidaemia and mild elevations in serum glutamic-oxaloacetic transaminase.[8] Fatty accumulation in liver and swelling of mitochondria are documented pathological changes. Thus, octanoate infusion appears to produce an experimental model of Reye's syndrome. Injections of other short-chain fatty acids produce milder symptomatology.[14]

The present study demonstrates that the short chain fatty acids propionate, butyrate, valerate and octanoate, and to a lesser extent isovalerate, also produce significant intracranial pressure elevations during continuous intravenous infusion into rabbits. This effect is produced with fatty acid concentrations of the same order of magnitude as those documented in patients with Reye's syndrome. Propionate concentrations rose to 27·15 μM/l; mean serum propionate concentrations in patients with Reye's syndrome is 14·89 μM/l.[7] Likewise, octanoate concentrations in patients range from 200-500 μM/l.[6] In rabbits serum concentrations rose to 500 μM/l during octanoate infusion.

Toxicity of the short chain fatty acids increased with increasing chain length. This corroborates other studies demonstrating a relationship between

chain length and toxicity. Zoborowsky *et al*[15] found that short chain fatty acids produced mitochondrial swelling *in vitro*. This effect was seen best with octanoate, the longest chain compound studied. Samson *et al*[16] demonstrated that intraperitoneal injections of short chain fatty acids produced coma in rats, and also found that coma was induced more easily as acids of increasing chain length were injected. Isovalerate, the only branched-chain short chain fatty acid studied, produced little change in intracranial pressure at similar concentrations, but did raise the pressure when the concentration was increased. This is of interest for two reasons. First, isovalerate is structurally related to valproic acid, a drug recently added to the list of anticonvulsant medications. One question which arises from the present study is whether valproic acid at high concentrations might produce intracranial pressure elevations and toxic effects by that mechanism. The second point of interest is that children with isovaleric acidaemia, an inborn error of leucine metabolism, have chronic elevations of serum isovaleric acid concentrations yet do not have obvious symptoms of intracranial pressure elevation; but when given a leucine load, these children develop vomiting and lethargy[17] which might correspond to changes in intracranial pressure.

The mechanism by which the short chain fatty acids produce intracranial pressure elevations has yet to be determined. Experimental studies suggest that they may alter blood-brain barrier permeability.[18] In addition, they inhibit cerebral Na^+K^+ ATPase activity[19 20] and may thus alter normal neuronal membrane ionic equilibrium. Whatever the mechanism of intracranial pressure elevation, a search for ways to clear short-chain fatty acids from the circulation may result in more effective treatment of the increased intracranial pressure in patients with Reye's syndrome.

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