A STUDY ON THE NARCOTIC ACTION OF THE SHORT CHAIN FATTY ACIDS [1]

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That the short chain fatty acids have an inhibitory action on many metabolic reactions has been shown in baker's yeast (1), bacteria (2–4), fungi (5), cell-free yeast extracts (1), and mammalian muscle (6). However, the effects of the short chain fatty acids on "intact" animals have not been studied in similar detail, except for a few studies on the toxicity of butyrate (7), β-hydroxybutyrate (8, 9), acetone (10) and acetoacetate (11). The present work is a study of the narcotic action of the neutralized salts of the short chain fatty acids upon "intact" rats.

METHODS

Solutions of the fatty acid salts were prepared daily by adding a weighed amount of the acid (Fisher Scientific Co.) to distilled water and neutralizing it to a pH of 7.4 with 20 per cent w/v NaOH. The pH was determined with a glass electrode pH meter while air bubbled through the solution to insure adequate mixing. In this connection, pH determinations are subject to error because these acids tend to form two phase systems and colloidal gels. In the present experiments care was taken to neutralize all the free fatty acid and only homogeneous preparations were used. The concentration of the fatty acid anion is specified in the individual cases. The β-hydroxybutyrate was purchased as the sodium salt (Nutritional Biochemical Corp.) and solutions were prepared by dissolving a weighed amount in distilled water and bringing it to a pH of 7.4.

The rats used in these experiments were females from a Sprague-Dawley strain maintained on Nutrena® dog food nuggets and weighing between 50 and 200 grams. They were fasted 24 hours before experimentation and weighed immediately prior to the fatty acid injection. For species comparisons, mice, guinea pigs, dogs, chicks and frogs were used; however, detailed data were collected only with rats.

Nephrectomized rats were prepared according to the directions of Farris and Griffith (12). With the animal under ether anesthesia a dorsal midline incision was made, the renal connections isolated and tied off at the hilum, then the kidney excised. With the operation concluded, the incision was closed by two or three skin sutures and the area sealed with collodion. Rats were made alloxan-diabetic by a tail vein injection of alloxan monohydrate (Nutritional Biochemical Corp.), 40 mg. per Kg. body weight. Two weeks later, the animals were fasted 24 hours and then urine samples collected for glucose analysis. Only those rats with glucose in the urine greater than 1 per cent were considered alloxan-diabetic.

To determine whether or not an animal was conscious the animal was placed on its back at one minute intervals after the fatty acid solution was injected. If it did not right itself within ten seconds, it was judged unconscious. With most animals, and rats in particular, this gives a reproducible end point.

Urine analyses for glucose and acetone were carried out with commercially obtained tablets (Ames Co.). The determination of the fatty acids in the urine was done chromatographically (13).

RESULTS AND DISCUSSION

Intravenous or intraperitoneal injection of the sodium salts of the short chain fatty acids produces unconsciousness in rats, frogs, chicks, mice, dogs and guinea pigs. The effect of various amounts and concentrations of the short chain fatty acids given intraperitoneally in rats is presented in Table I. As can be seen, unconsciousness occurs in two to forty minutes after injection and may persist for as long as an hour. The amount of fatty acid anion which will produce unconsciousness in 50 per cent of a sample of rats (E_{50}) was determined by plotting the per cent of each group of animals that lost consciousness against the amount injected and then selecting the amount which corresponded to 50 per cent (14).

There is a definite relationship between the amount of the fatty acid which will produce unconsciousness and the carbon chain length of the compound. As might be expected, the E_{50} decreases rapidly with the increase in chain length (Figure 1).

It should be noted that the E_{50} for a given fatty acid depends upon the concentration of the acid as well as its chain length. This point is demonstrated in Figure 2 where the E_{50} for octanoate

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TABLE I

The effect of the intraperitoneal injection of the sodium salts of the short chain fatty acids

Rat no.	Fatty acid	Conc. *M*	Amount injected *mM/Kg.*	Response	Time until unconscious *minutes*	Duration unconscious *minutes*	E_{50} *mM/Kg.*
1	Acetate	1.0	20	None			
2			21	None			
3			30	None			
4			33	None			
5			36	None			
6		2.0	56	Unconsc.	18	15 (died)	
7	Propionate	1.0	28	None			
8			28	None			
9			28	None			
10			28	Unconsc.	18	18	
11			28	Unconsc.	20	36	
12			30	None			
13			30	None			29.0
14			30	Unconsc.	20	4	
15			30	Unconsc.	20	4	
16			30	Unconsc.	18	18	
17	Butyrate	1.0	12.5	None			
18			12.5	None			
19			12.5	None			
20			12.5	None			
21			12.5	None			
22			14.0	None			
23			14.0	None			
24			14.0	None			
25			14.0	Unconsc.	11	2	14.2
26			14.0	Unconsc.	11	3	
27			16.0	Unconsc.	14	6	
28			16.0	Unconsc.	8	20	
29			16.0	Unconsc.	8	21	
30			16.0	Unconsc.	7	11	
31			16.0	Unconsc.	7	20	
32			20.0	Unconsc.	11	5	
33			20.0	Unconsc.	9	51	
34			20.0	Unconsc.	8	40	
35			20.0	Unconsc.	7	50	
36			20.0	Unconsc.	6	42	
37		0.5	20.0	None			
38			20.0	None			
39			20.0	None			
40			20.0	None			
41			20.0	None			
42			23.0	None			
43			23.0	None			23.0
44			23.0	Unconsc.	24	2	
45			23.0	Unconsc.	14	12	
46			26.0	Unconsc.	14	1	
47			26.0	Unconsc.	12	5	
48			26.0	Unconsc.	12	7	
49			26.0	Unconsc.	11	9	
50	Valerate	0.67	18	None			
51			18	None			
52			18	Unconsc.	32	6	
53			18	Unconsc.	22	28	
54			18	Unconsc.	22	22	18.0
55			18	Unconsc.	22	26	
56			18	Unconsc.	22	30	
57			18	Unconsc.	21	44	
58			18	Unconsc.	20	28	
59		0.50	18	None			
60			18	None			
61			18	None			
62			18	None			

TABLE I—*Continued*

Rat no.	Fatty acid	Conc. *M*	Amount injected *mM/Kg.*	Response	Time until unconscious *minutes*	Duration unconscious *minutes*	E_{50} *mM/Kg.*
63	Valerate		18	Unconsc.	28	4	19.0
64			20	None			
65			20	None			
66			20	Unconsc.	38	10	
67			20	Unconsc.	18	30	
68			20	Unconsc.	16	16	
69			22	Unconsc.	24	8	
70			22	Unconsc.	16	8	
71			22	Unconsc.	14	44	
72			22	Unconsc.	12	32	
73	Caproate	0.50	12	None			
74			12	None			
75			12	None			
76			12	None			
77			12	Unconsc.	9	2	15.0
78			14	None			
79			14	None			
80			14	None			
81			14	None			
82			14	Unconsc.	8	6	
83			15	None			
84			15	None			
85			15	Unconsc.	9	5	
86			15	Unconsc.	8	4	
87			15	Unconsc.	6	6	
88			16	Unconsc.	9	7	
89			16	Unconsc.	7	7	
90			16	Unconsc.	6	10	
91			16	Unconsc.	5	9	
92			16	Unconsc.	4	10	
93	Heptanoate	0.50	5	None			
94			5	None			
95			5	None			
96			5	None			
97			5	None			
98			6	None			
99			6	None			
100			6	None			
101			6	Unconsc.	5	5	6.2
102			6	Unconsc.	5	6	
103			7	None			
104			7	Unconsc.	5	5	
105			7	Unconsc.	5	6	
106			7	Unconsc.	5	7	
107			7	Unconsc.	6	7	
108	Octanoate	1.0	2	None			
109			2	None			
110			2	Unconsc.	4	4	
111			2	Unconsc.	3	6	2.0
112			2	Unconsc.	2	10	
113		0.5	3.4	None			
114			3.4	None			
115			3.4	None			
116			3.4	None			
117			3.4	None			
118			3.6	None			
119			3.6	None			
120			3.6	None			3.7
121			3.6	Unconsc.	5	2	
122			3.6	Unconsc.	5	3	
123			3.8	None			
124			3.8	None			
125			3.8	Unconsc.	5	3	
126			3.8	Unconsc.	5	5	
127			3.8	Unconsc.	5	5	

TABLE I—*Continued*

Rat no.	Fatty acid	Conc. *M*	Amount injected *mM/Kg.*	Response	Time until unconscious *minutes*	Duration unconscious *minutes*	E_{50} *mM/Kg.*
128	Octanoate		5.0	Unconsc.	5	7	
129			7.5	Unconsc.	3	32	
130		0.1	6.0	Unconsc.	11	12	
131			6.0	Unconsc.	10	14	
132			6.0	Unconsc.	9	22	
133			6.0	Unconsc.	8	>32	
134			6.0	Unconsc.	7	>32	
135	Pelargonate	0.3	2.5	None			
136			2.5	None			
137			2.5	None			
138			2.5	None			
139			2.5	None			
140			2.7	None			
141			2.7	None			2.8
142			2.7	None			
143			2.7	None			
144			2.7	Unconsc.	8	2	
145			3.0	Unconsc.	5	4	
146			3.0	Unconsc.	4	8	
147			3.0	Unconsc.	3	6	
148			3.0	Unconsc.	3	10	
149			3.0	Unconsc.	3	10	
150	Caprate	0.1	2.8	None			
151			2.8	None			
152			2.8	None			
153			2.8	None			
154			2.8	None			
155			3.2	None			
156			3.2	None			
157			3.2	None			3.3
158			3.2	None			
159			3.2	Unconsc.	14	2	
160			3.4	None			
161			3.4	None			
162			3.4	Unconsc.	7	1	
163			3.4	Unconsc.	4	4	
164			3.4	Unconsc.	4	6	
165			3.6	Unconsc.	7	1	
166			3.6	Unconsc.	5	3	
167			3.6	Unconsc.	5	3	
168			3.6	Unconsc.	5	6	
169			3.6	Unconsc.	7	6	

as a function of concentration is presented. This pronounced effect of concentration upon the E_{50} is probably a result of a rapid disposal of the fatty acid anion and the influence of concentration upon the rate of absorption from the injection site. With propionate, solutions of low concentration are not effective enough to be adequately studied, whereas with pelargonate and caprate, only solutions of low concentration are sufficiently homogeneous to give reliable results. Therefore, the action of the three, nine and ten carbon members (propionate, pelargonate, and caprate respectively) cannot be quantitatively compared. However, their effectiveness corresponds with the general pattern as seen in Table I.

At the two carbon chain length the narcotic action becomes so weak that large quantities of acetate can be administered without the characteristic effect exhibited by the other acids. The injection of about 56 millimoles per Kg. body weight of 2 M sodium acetate will cause unconsciousness, but the pattern of the response is different. Following the injection of the other fatty acids there is reduced muscular tone and activity, whereas after the injection of the sodium acetate severe muscular spasms and convulsive movements occur. It is interesting to note that the intraperitoneal injection of the same amount (56 millimoles per Kg.) of 2 M NaCl produces the same type of response: that is, a few minutes after the injection the rat

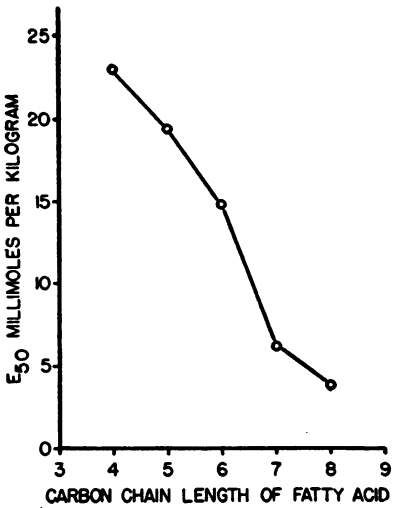

FIG. 1. E_{50} OF FATTY ACID ANION AS A FUNCTION OF CHAIN LENGTH

All acids neutralized to pH 7.4; conc. 0.5 M; given intraperitoneally. Data taken from Table I.

shows muscular twitching which becomes progressively increased until severe muscular spasms cause the animal to thrash about violently. The animal loses consciousness and dies, usually within an hour after the injection. The similarity of the response from the 2 M sodium acetate injection to that from the 2 M NaCl suggests that the mechanism of action here is related to the sodium ion rather than the acetate anion. This effect of high sodium ion has been reported by Ulrich and Shternov (15).

As might be expected, the length of time from injection until unconsciousness, the E_{50}, and the length of time of unconsciousness also depend to some extent upon the rate of absorption from the injected site. Consequently, subcutaneous administration is not very effective and nothing more than a sluggishness occurs. Further, the forced feeding of as much as twice the E_{50} will not even produce sluggishness. On the other hand, when the compounds are given intravenously unconsciousness occurs within a few seconds after the injection and with somewhat less than half of the amount necessary to cause unconsciousness with the intraperitoneal route (Table II).

Hemolysis

As it is common knowledge that washed red blood cells will hemolyze when suspended in a sufficiently concentrated solution of the fatty acid anions (16), it seemed reasonable to consider what part hemolysis might play in the reactions which follow fatty acid injection. Accordingly, we have drawn blood by cardiac puncture from rats made unconscious from a fatty acid injection and examined it grossly after centrifugation: in no instance was hemolysis noted.

Kidneys

The fatty acids and some of their metabolic products appear in the urine following their injection (Table III). From these findings we reasoned that the recovery from the narcotic action of the compounds rested mainly in excretion by the kidneys. But this idea was not supported by experiments on nephrectomized rats. The E_{50} and the length of time until recovery in nephrectomized rats seem to be of the same order of magnitude as in the control rats (compare Table IV with Table I).

Influence of (OH) group in carbon chain

To determine if the introduction of a hydroxyl group into the carbon chain would alter the action, we injected lactate and β-hydroxybutyrate and compared their effectiveness with the corresponding fatty acids, propionate and butyrate. The results show that the hydroxy compounds are much less effective (Table V): In fact, the E_{50} for 1 M lactate and β-hydroxybutyrate could not be de-

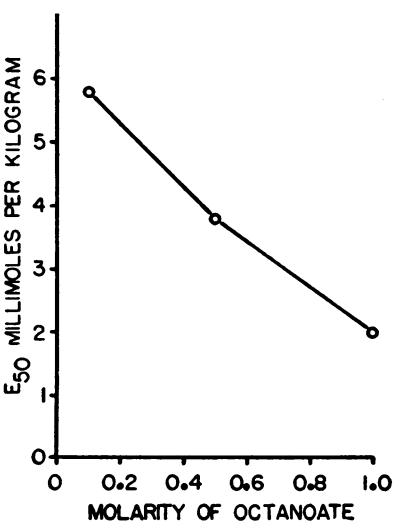

FIG. 2. E_{50} OF SODIUM OCTANOATE AS A FUNCTION OF CONCENTRATION

pH 7.4; given intraperitoneally. Data taken from Table I.

TABLE II

The effect of the injection site on the response to the sodium salts of the fatty acids

Rat no.	Injection site	Fatty acid	Conc. *M*	Amount injected *mM/Kg.*	Response	Time until unconsc.	Duration of unconsc.
170	Intravenous	Propionate	1.0	12.3	None		
171	(tail vein)			18.0	Unconsc.	5 sec.	Died
172				19.0	Unconsc.	5 sec.	25 sec.
173		Valerate	1.0	2.0	None		
174				8.8	None		
175				10.0	Unconsc.	<1 min.	2 min.
176				12.0	Unconsc.	<1 min.	2 min.
177		Caproate	0.5	5.6	None		
178				6.2	Unconsc.	30 sec.	3 min.
179		Heptanoate	0.4	1.6	None		
180				2.0	Unconsc.	<30 sec.	<30 sec.
181				2.0	Unconsc.	5 sec.	55 sec.
182		Octanoate	0.5	0.8	Unconsc.	5 sec.	40 sec.
183				0.8	None		
184				1.0	Unconsc.	5 sec.	1 min.
185				1.2	Unconsc.	20 sec.	1 min.
186				1.5	Unconsc.	5 sec.	3 min.
187		Pelargonate	0.3	0.5	None		
188				0.5	None		
189				0.7	Unconsc.	5 sec.	
190				0.7	Unconsc.	5 sec.	160 sec.
191				0.7	Unconsc.	5 sec.	6 min.
192		Caprate	0.1	0.5	None		
193				0.6	Unconsc.	20 sec.	1 min.
194				0.8	Unconsc.	10 sec.	95 sec.
195	Subcutaneous	Valerate	1.0	22.0	None		
196	Gastro-	Valerate	1.0	22.0	None		
197	intestinal			22.0	None		
198	(forced			26.0	None		
199	feeding)			35.0	None		

termined because a definitive state of unconsciousness did not occur. Instead, convulsions similar to those seen after the injection of NaCl discussed earlier resulted. With 1 M β-hydroxybutyrate these convulsions occurred after the administration of about 40 millimoles per Kg. body weight, whereas the E_{50} of 1 M butyrate is 14.2 millimoles per Kg. body weight.

TABLE III

Urine analysis following intraperitoneal injection of the fatty acid salts *

Fatty acid injected	Urine analysis		
	Fatty acid %	Glucose %	Ketones %
Propionate (30 *mM/Kg.*)		1.0	0.05
Butyrate (28 *mM/Kg.*)	0.2–0.3	0.0	2.0
Valerate (24 *mM/Kg.*)	0.4–0.7	1.0	0.0
Caproate (13 *mM/Kg.*)	0.2	0.0	2.0
Octanoate (3.9 *mM/Kg.*)	0.0 Octanoate 0.1 Butyrate		

* Rats fasted 24 hours before experiment. Urine collected for 6 to 12 hours after injection.

Alloxan-diabetic rats

Although the alloxan-diabetic rat does not develop ketosis as is seen in severe human diabetes (17), there is some disturbance of fat metabolism in alloxan diabetes (18) thus it seemed worthwhile to ascertain the susceptibility of alloxan-diabetic rats to the narcotic action of the fatty acid anions. The results of these experiments are given in Table VI. It is clear from these data that the alloxan-diabetic rats were actually less susceptible than normal rats.

Mechanism of action

The narcotic actions studied here are probably a direct action of the fatty acid anion on the cen-

TABLE IV

The effect of intraperitoneal injection of sodium butyrate and sodium valerate on nephrectomized rats

Rat no.	Fatty acid	Conc. *M*	Amount injected *mM/Kg.*	Response	Time until unconsc. *minutes*	Duration of unconsc. *minutes*
200	Butyrate	0.95	15.0	None		
201			15.0	None		
202			20.0	Unconsc.	11	10
203	Valerate	1.0	19.5	None		
204				None		
205				Unconsc.	28	4
206				Unconsc.	24	2
207				Unconsc.	24	30
208				Unconsc.	18	10
209				Unconsc.	18	22
210				Unconsc.	9	60

tral nervous tissue. The electroencephalographic changes which occur during the unconscious state support this idea (19). The fatty acid salts may inhibit the metabolic activity of cerebral tissue as they do muscle (6) and yeast (1). Several properties characterize their inhibition of yeast cell metabolism: 1. A nonspecificity, as evidenced by the large number of reactions affected, 2. An increasing action with an increase in chain length, 3. A reduction of the inhibitory action when a hydroxyl or carboxyl group is introduced into the hydrocarbon part of the molecules, 4. A reversibility.

The similarity of these properties with those demonstrated in the present study is certainly striking. However, this similarity does not necessarily mean that the cellular mechanism of action is the same in the two situations.

SUMMARY

Injection of the neutralized short chain fatty acids will produce unconsciousness in experimental animals. The amount of the fatty acid which will produce this response decreases with an increase in chain length. Further, the amount which will produce a loss of consciousness for a given fatty acid depends upon the concentration and the site of injection. The introduction of an (OH) group into the carbon chain reduces the narcotic action.

TABLE V

The effect of intraperitoneal injection of sodium lactate, sodium β-hydroxybutyrate and acetone on rats

Rat no.	Compound injected	Conc. *M*	Amount injected *mM/Kg.*	Reaction
211	Sodium	0.5	30	Sluggish movements, no loss of consciousness
212	lactate	1.0	30	Sluggish movements, no loss of consciousness
213			30	Sluggish movements, no loss of consciousness
214			34	Sluggish movements, no loss of consciousness
215			34	Sluggish movements, no loss of consciousness
216			36	Sluggish movements, no loss of consciousness
217			38	Sluggish movements, no loss of consciousness
218			38	Sluggish movements, no loss of consciousness
219	Sodium	0.5	30	No loss of consciousness
220	β-hydroxy	1.0	30	No loss of consciousness
221	butyrate		40	Severe muscular spasms and death in 57 min.; righting reflexes present until death.
222			50	Severe muscular spasms; righting reflexes present until death in about one hour.
223	Acetone	1.0	7	No loss of consciousness
224			37	No loss of consciousness
225			40	No loss of consciousness
226			50	No loss of consciousness
227			60	No loss of consciousness
228			80	Loss of consciousness for five minutes, approximately four minutes after injection.

TABLE VI

The effect of intraperitoneal injection of sodium valerate on alloxan-diabetic rats

Rat no.	Fatty acid	Conc. *M*	Amount injected *mM/Kg.*	Response	Time until unconsc. *minutes*	Duration of unconsc. *minutes*
229	Valerate	0.5	18	None		
230				None		
231				None		
232				None		
233			20	None		
234				None		
235				None		
236				Unconsc.	20	6
237			22	None		
238				None		
239				Unconsc.	28	4
240				Unconsc.	16	20
241			24	Unconsc.	38	2
242				Unconsc.	22	6
243				Unconsc.	16	12

There is no hemolysis from the amount of fatty acid which will produce unconsciousness. Nephrectomy does not seem to alter the duration of the narcotic action.

It is suggested that the mechanism of action at the cellular level may be the same as that in the fatty acid inhibition of yeast metabolism.

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