THE ACTION OF SODIUM FLUOROACETATE ON INTESTINAL SMOOTH MUSCLE

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Rona and Neukirch (1, 2, 3) have shown that segments of rabbit small intestine, when placed in glucose free Tyrode solution under aerobic conditions, undergo a gradual decrease in the amplitude of spontaneous contractions and a reduction in tone. The addition of a number of sugars, fatty acids and metabolic intermediates resulted in the restoration of the tone and amplitude of spontaneous contractions (1-3). These effects have been confirmed by Feldberg and Solandt (4) and Furchgott and Shorr (5). The restoring properties of these substrates are probably due to their utilization as sources of energy for muscle contraction (1-5). As pointed out by Furchgott and Shorr (5) this method of Rona and Neukirch has certain advantages over the more conventional methods for determining substrate utilization.

Enzyme inhibitors such as iodoacetate, phlorhizin and glyceraldehyde can block the response of depleted intestinal strips to the addition of various active substrates (6). It has been shown that sodium fluoroacetate (NaFAc) is a powerful inhibitor of enzyme systems which catalyze the utilization of pyruvate, acetate, fatty acids (7-9) and fumarate (10). In the present study the action of NaFAc on intestinal smooth muscle in the presence of different substrates and in conjunction with several inhibitors was determined and attempts have been made to interpret these results in the light of modern concepts of intermediary metabolism.

MATERIALS AND METHODS. Rabbits weighing 2 to 3 kgm. were killed by a blow on the neck and 10 to 15 cm. of the upper portion of the intestine were removed, washed and placed in glucose-free Tyrode solution. In a number of animals the lowest portions of the ileum and the rectum were removed and treated in a similar manner. Pieces of gut about 2 cm. in length were suspended in a smooth muscle bath kept at 38° C. The movements of the longitudinal muscles were recorded on a kymograph by means of a Gimble lever or frontal writing lever as modified by Schild (11).

The bathing fluid employed was Tyrode solution (12), and the glucose in this solution was either omitted or substituted for by various other substrates. The gas mixtures used for aerating the bath were either 95 per cent O2 and 5 per cent CO2 or 95 per cent N2 and 5 per cent CO2. Sodium fluoroacetate* and the substrates employed were dissolved in glucose-free Tyrode solution. All solutions were neutralized by means of dilute sodium hydroxide to a neutral pH. In a number of experiments the phosphate and magnesium ions in Tyrode solution were omitted. The effect of these modified solutions on the response of intestinal strips to various substrates and NaFAc was studied.

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2 Kindly supplied by the Fish and Wildlife Service, Denver, Colorado.
RESULTS. The effects of NaFAc on intestinal strips were qualitatively and quantitatively different when different substrates were used. In the present study glucose, acetic acid, pyruvic acid and butyric acid were used as substrates either alone or in combination.

The action of NaFAc in the presence of glucose: Strips of the upper portions of the intestine were suspended in Tyrode solution containing 5.5 mM glucose (1 gm. per l.). These continued to contract for several hours without any appreciable change in the rate or height of spontaneous contractions. The addition of NaFAc resulted in a primary increase followed by a decrease in tone and a reduction in amplitude of these contractions. These changes were in turn followed by a decrease in the rate and an alteration in the character of the contractions. The latter phenomenon was characterized by irregular contractions which somewhat resembled those of the isolated large intestine and have been designated the fluoroacetate resistant contractions (fig. 1, upper tracing). The minimal effective concentration of NaFAc was 0.02 mM. An increase of this concentration to 10 mM did not abolish the irregular spontaneous contractions described above.

The action of NaFAc in the presence of sodium acetate: Strips of the upper portion of the small intestine were suspended in Tyrode solution containing sodium acetate (5.5 mM) instead of glucose. These strips contracted spontaneously with little change either in amplitude or rate of spontaneous contractions for several hours, provided the strip was not washed. Repeated washing with sodium acetate-containing Tyrode solution resulted in a general decrease in the height of spontaneous contractions. In the present study the strips were suspended in the bath and the original solution was not changed throughout the experiment. Addition of NaFAc (0.1–0.2 mM) to a bath containing sodium acetate as substrate resulted in complete loss of spontaneous activity and a reduction in tone (fig. 1, lower tracing). In no instance did we observe the appearance of fluoroacetate resistant contractions when sodium acetate was the only substrate present. With pyruvate, butyrate, caproate, acetoacetate, or oxalacetate as substrates the results were the same as with acetate. This action of NaFAc was in marked contrast to that observed when glucose was used as the substrate.

The effect of NaFAc on the depleted intestinal strip: The method of Rona and Neukirch (1, 2) was utilized in a number of experiments. Upper intestinal strips were suspended in glucose-free Tyrode solution and within 30 to 60 minutes the height of the spontaneous contractions was reduced by about 70 to 80 per cent. Addition of various substrates following the addition of NaFAc (1.6 mM) showed that only glucose and mannose were able to bring about an increase in the height of the spontaneous movements which resembled those contractions previously designated as fluoroacetate resistant contractions. Acetate, pyruvate, butyric, caproic, caprylic, succinic, fumaric and alpha-ketoglutaric acids did not show any action on spontaneous activity in the fluoroacetate poisoned and depleted intestinal strip.

It has been shown previously that acetate and pyruvate can protect tissue slices and the isolated heart against the action of sodium fluoroacetate (9, 13).
Results obtained with different concentrations of NaAc show that the higher the NaAc concentration the greater the concentration of NaFAc needed to produce 50 per cent and 100 per cent inhibition of the spontaneous contractions of the small intestine. These data are compatible with the interpretation that NaFAc competes with sodium acetate for the enzyme system responsible for the oxidation of acetate.

Fig. 1. The effect of sodium fluoroacetate on the spontaneous contractions of the upper portion of the isolated rabbit intestine.

Upper Tracing. Tyrode solution containing 5.5 mM glucose. At the mark 1.6 mM NaFAc (16 mgm. per cent) was added to the bath.

Lower Tracing. Tyrode solution containing 5.5 mM sodium acetate. S denotes stoppage of the drum for five minutes. At the mark 0.05 mM NaFAc was added to the bath.

Time in minutes.

The quantitative action of NaFAc in the presence of various substrates: Various portions of the small intestine showed quantitative differences in their response to NaFAc. It was thus essential to use only a limited part of that organ for any type of quantitative work. In the experiments to be described the upper 10 to 15 cm. of the small intestine were used. The relative inhibitory action of NaFAc on spontaneous contractions was determined for glucose, sodium acetate (NaAc), sodium pyruvate and sodium butyrate separately and for a combination of sodium acetate and glucose. Figure 2 summarizes the results obtained, when
concentrations of NaAc and glucose were used which had about the same effect in sustaining spontaneous contractions. These concentrations were observed to be 1.4 and 5.5 mM for NaAc and glucose, respectively. In the presence of 1.4 mM NaAc alone, inhibition of spontaneous contractions was complete when the concentration of NaFAc reached 0.004 mM. With glucose as substrate spontaneous activity was not inhibited more than 80 per cent regardless of the concentration of sodium fluoroacetate. A combination of NaAc and glucose resulted in a definite increase of resistance of these strips to NaFAc and spontaneous activity could not be completely abolished regardless of the concentration of NaFAc.

![Graph](image)

**Fig. 2.** Percentage inhibition of the spontaneous contractions of the upper portions of the rabbit intestine by fluoroacetate in the presence of acetate or glucose.

- ●● Tyrode solution containing 1.4 mM sodium acetate instead of glucose
- ○○ Tyrode solution containing 5.5 mM glucose
- ■■ Tyrode solution containing 5.5 mM glucose and 1.4 mM acetate.

Each point on the curves represents the average of eight to twelve individual experiments.

The effects of some other substrates on the fluoroacetate inhibition were studied. Either sodium acetate, pyruvate or butyrate was used as substrate in concentration of 5.5 mM. The results are given in fig. 3 and it can be seen that with all three substrates the percentage inhibition of the spontaneous contractions produced by NaFAc was about the same. With none of these substrates was it possible to observe fluoroacetate resistant contractions.

The time for the production of maximal effects is dependent on the concentration of NaFAc and the type of concentration of the substrate used. Figure 4A shows that with an increase in the NaFAc concentration the time for producing maximal effects is reduced. Furthermore, in the presence of glucose as substrate, maximal effects occur much more rapidly than in the presence of acetate or butyrate (figure 4A). When the NaFAc concentration is kept constant the concentration of sodium acetate affects the time for the production of the maximal effects. Figure 4B summarizes these results and it can be seen that an increase in the substrate concentration also increases the time for the production of maximal effects.
Fig. 3. Percentage inhibition by NaFAc of the spontaneous contractions of the upper portions of the rabbit intestine in the presence of various substrates.

- ○ 5.5 mM sodium acetate
- ● 5.5 mM sodium pyruvate
- ○ 5.5 mM sodium butyrate

Ordinate: Percentage inhibition
Abscissa: Concentration (mM) of sodium fluoroacetate.

Fig. 4A. The effect of the concentration of NaFAc on the time for the production of maximal effects. Upper isolated intestinal segments suspended in Tyrode solution.

- ● Glucose 5.5 mM
- ○ Sodium acetate, 5.5 mM
- ● Sodium butyrate, 5.5 mM

Ordinate: Time in minutes for the production of maximum inhibition of the spontaneous contractions.
Abscissa: Concentration of NaFAc (mM).

Fig. 4B. The influence of sodium acetate concentration on the time for the production of 95–100 per cent inhibition of the spontaneous contractions by sodium fluoroacetate (0.8 mM). Upper isolated intestinal segments suspended in Tyrode solution.

Ordinate: Time in minutes for producing a maximum inhibition
Abscissa: Concentration of sodium acetate (mM).
Reversibility of the NaFAc effect. In the presence of substrate (glucose, acetate, pyruvate or butyrate) and under aerobic conditions the effects of NaFAc on spontaneous and on the acetylcholine contractions cannot be reversed by washing. Furchgott (14) has shown that under anaerobic conditions this irreversible effect does not occur since washing of the intestinal strip caused the reappearance of normal intestinal contractions when aerobic conditions were resumed. These results of Furchgott were confirmed in this laboratory.

The time necessary for producing the irreversible effect under aerobic conditions was studied using a 1 mM concentration of NaFAc and 5.5 mM NaAc as substrate. Figure 5 summarizes these results and it can be seen that irreversible effects are attained after an exposure lasting eight to ten minutes. The percentage reduction in amplitude is related to the time of exposure (figure 5).

Fig. 5. The effect of time of exposure of isolated upper intestinal strips to sodium fluoroacetate (1 mM). Each point on the curve represents six to eight individual experiments.

In the experiments where exposure to NaFAc lasted eight to twelve minutes minimal effects were observed at the time of washing which continued to progress to maximal effects. It is possible that within eight to ten minutes NaFAc was fixed and its full effect only became apparent after the exhaustion of intermediates which are not sensitive to NaFAc but are not replenished in the NaFAc poisoned muscle.

The fluoroacetate resistant contractions: It has been shown above that in the presence of glucose, NaFAc was unable to inhibit completely spontaneous contractions. These fluoroacetate resistant contractions are dependent upon the presence of glucose in the bathing fluid. Removal of glucose by washing with the glucose-free Tyrode solution abolished the spontaneous contractions (figure 6). The addition of sodium acetate did not restore these contractions, whereas glucose addition resulted in a prompt recovery. Experiments conducted in a similar manner have shown that in the presence of NaFAc the addition of lactate, pyruvate, butyrate, caproate, succinate, fumarate, alpha-ketoglutarate and oxalacetate, as well as galactose, fructose, and lactose are unable to restore these residual spontaneous contractions. Besides glucose, mannose is the only sub-
strate so far studied which has the ability to restore spontaneous contractions in the NaFAC-poisoned intestine. The degree of this restoration is about equal for glucose and mannose.

Results obtained by Prasad (15), Furchgott and Shorr (16) and in this laboratory (17) indicate that intestinal strips of the rabbit can utilize anaerobically glucose and mannose for muscle contraction. The effect of anaerobiosis on the fluoroacetate resistant contractions was determined by a change of the aerating gas from oxygen to nitrogen. This change resulted in a reduction or complete stoppage of spontaneous contractions lasting five to fifteen minutes and then was followed by the reappearance of spontaneous contractions. It is concluded that anaerobiosis modifies but does not abolish the fluoroacetate resistant contractions in smooth muscle of the rabbit intestine.

The removal of phosphate and magnesium ions from the Tyrode solution did not substantially modify the fluoroacetate resistant contractions.

The effect of a number of enzyme inhibitors on the fluoroacetate resistant contractions was studied. It could be shown that sodium azide (1.3–1.5 mM), dinitrophenol (0.1–0.2 mM), iodoacetic acid (0.05–0.1 mM), and p-chloromercuribenzoate (0.14–0.28 mM) completely inhibited the fluoroacetate resistant spontaneous contractions while malonate and propionate were ineffective in 0.01 M concentrations. Sodium cyanide (2–4 mM) inhibited the fluoroacetate resistant contractions for about five to fifteen minutes. This inhibition was followed by a gradual recovery of these contractions. This action of cyanide is
very similar to that observed when anaerobiosis was superimposed on sodium fluoroacetate resistant contractions by means of nitrogen.

**Action of NaFAc on different portions of the intestine:** As stated previously the experiments so far described were conducted on the upper 10–15 cm. of the small intestine. In a number of experiments ileal and rectal strips were exposed to NaFAc. Ileal strips showed a loss of tone and the regular spontaneous contractions in the presence of 1.6 mM NaFAc. However, the fluoroacetate resistant spontaneous contractions of the ileum were stronger and more pronounced than those of the upper intestine. Rectal strips showed only minimal changes following the addition of NaFAc (1.6–3.2 mM). These changes were characterized by a slight slowing of the spontaneous contractions and some reduction in tone. It was apparent that in the presence of glucose the spontaneous contractions of the rectum were more resistant to NaFAc than those of the ileum or upper intestine.

**The action of NaFAc on circular muscle contractions:** Circular muscle contractions were recorded by the Trendelenburg technic (18). The pressure in the lumen was 3–5 cm. of saline and both circular and longitudinal contractions were recorded by adequate levers. Though no quantitative data were obtained, no essential qualitative differences in response to NaFAc between circular and longitudinal muscle could be observed.

**The acetylcholine response in the presence of NaFAc:** In the presence of glucose as substrate acetylcholine produces a sustained contraction in unpoisoned intestinal smooth muscle. In the presence of NaFAc (1.6 mM) the acetylcholine response is changed: addition of acetylcholine (20–100 microgm. per cent) results in rapid contractions which are not sustained for more than 20 to 30 seconds. If spontaneous contractions are present the acetylcholine contracture is followed by an inhibition of these spontaneous contractions of the longitudinal muscle. Washing out the glucose by means of glucose-free Tyrode solution results in the loss of the ability of the strip to respond to acetylcholine. However, on the addition of glucose or mannose to the washed strip, the response to acetylcholine is restored (fig. 7). Pyruvate, acetate, fatty acids, succinate, fumarate, alpha-ketoglutarate and lactate are unable to restore this response to acetylcholine. Furthermore, the enzyme inhibitors, sodium azide (1.5–3 mM), dinitrophenol (0.11 mM), and iodoacetate (0.055–0.11 mM) will inhibit the acetylcholine response of longitudinal muscle of the NaFAc poisoned intestinal strip. Removal of the phosphate and magnesium ions from the Tyrode solution did not have any observable effect on the acetylcholine response, either in normal or NaFAc-poisoned intestinal strips. The height of the fluoroacetate resistant acetylcholine contraction was somewhat reduced under anaerobic conditions but was never completely inhibited by this condition as long as glucose or mannose was present in the bath. In a similar manner sodium cyanide (2–4 mM) reduced but never abolished the acetylcholine response.

**Response to epinephrine and atropine:** The NaFAc resistant spontaneous movements were inhibited by epinephrine (10 microgm. per cent). This effect could be washed out and in general the response was similar to that observed
in normal intestinal strips. With atropine sulfate (0.5–1 mgm. per cent) the primary dose produced an inhibition lasting three to five minutes and was followed by the recovery of the spontaneous contractions. A second similar dose of atropine sulfate did not influence the NaFAc resistant contractions. These results are similar to those observed with movements of unpoisoned intestinal strips.

Discussion. In rabbit isolated intestinal strips, fluoroacetate sensitive and fluoroacetate resistant contractions can be demonstrated. Pyruvate, acetate and
some of the even numbered carbon fatty acids, though able to sustain con-
tractions in the normal muscle are unable to do so in the presence of a sufficient
concentration of NaFAc. The data obtained with different NaAc concentrations
show that the higher the acetate concentration the more NaFAc is necessary
to produce a certain degree of inhibition (table 1 and figure 4A). These findings
are in harmony with the interpretation that fluoroacetate acts as a competitive
inhibitor of enzyme systems necessary for acetate utilization.

The fluoroacetate resistant contractions have been demonstrated in the presence
of glucose or mannose. These results can be explained by the hypothesis that
-glucose or mannose can supply energy for contraction by two pathways. The
fluoroacetate sensitive pathway may include pyruvate and acetate as inter-
mediates. The fluoroacetate resistant pathway is partially resistant to anaero-
biosis, cyanide, malonate and propionate inhibition and is sensitive to dinitro-
phenol, iodoacetate and p-chloromercuribenzoate. Intestinal smooth muscle can

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<tr>
<th>Concentration of NaAc</th>
<th>Concentration of NaFAc producing 50 per cent reduction in contraction amplitude</th>
<th>Concentration of NaFAc producing 95-100 per cent inhibition of spontaneous contractions</th>
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<tr>
<td>mM</td>
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<tr>
<td>1.4</td>
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<td>0.18</td>
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<tr>
<td>5.5</td>
<td>0.02</td>
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utilize glucose and mannose anaerobically as a source of contraction energy
(15, 16). A comparison of this anaerobic pathway and the one resistant to
NaFAc indicates that they respond in a similar manner towards enzyme inhibi-
tors. It is thus possible that the fluoroacetate resistant pathway may be
related to the anaerobic one and that energy for contraction of NaFAc poisoned
intestinal smooth muscle can be obtained through anaerobic glycolysis.

Since the fluoroacetate resistant contractions were partially resistant to high
concentrations of cyanide or malonate, it is probable that neither the Krebs
cycle nor the cytochrome system is essential for this type of contraction.

The differences in response to NaFAc between the small intestine and rectum
are striking. Similar differences in response to anaerobiosis have been observed
in this laboratory. It is possible that the large intestine may have a more highly
developed anaerobic glycolytic path than the small intestine. The data also
indicate that the ileum is more resistant to anaerobiosis and NaFAc than the
duodenum. It is thus probable that we are dealing here with one of the many
metabolic gradients previously described for the gastrointestinal tract (19).

SUMMARY

Sodium fluoroacetate inhibits the spontaneous contractions of intestinal smooth
muscle. This effect is quantitatively and qualitatively different when different
types of substrate are used. Complete inhibition of spontaneous contractions
does not occur in the presence of glucose or mannose. Inhibition of spontaneous contractions is complete in the presence of acetate, pyruvate, oxalacetate, acetoacetic acid and a number of fatty acids as substrates.

Strips of the large intestine are more resistant to NaFAc than those of the small intestine.

The fluoroacetate resistant contractions occurring in the presence of glucose are abolished by iodoacetate, p-chloromercuribenzoate, dinitrophenol and sodium azide. They are not abolished by anaerobiosis, sodium cyanide, malonic and propionic acid. Under aerobic conditions and in the presence of substrate the effects of NaFAc are not reversed by repeated washing with Tyrode solution.

REFERENCES