Biochimica et Biophysica Acta, 433 (1976) 654–661

BBA 77307

THE EFFECT OF SHORT CHAIN FATTY ACIDS ON TRANSMURAL ELECTRICAL POTENTIAL ACROSS RAT SMALL INTESTINE IN VIVO

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(Received September 30th, 1975)
(Revised manuscript received January 27th, 1976)

SUMMARY

Short chain fatty acids suddenly produce a phasic increase in transmural electrical potential difference (PD) when placed in the lumen of rat small intestine in vivo. With concentrations of propionate ranging from 50 μM to 1000 μM the amplitude of the response in jejunum is about 5.5 mV. The concentration giving half this effect is about 20 μM. With 10 mM propionate the duration of the response is 3–5 min; after this, PD again equals the control value and the gut is refractory to further additions. Removing propionate from the mucosal surface produces no change in PD, but does restore responsiveness to subsequent exposure to short chain fatty acids.

This effect is independent of a variety of other alterations in PD such as those caused by sugars, amino acids, bile salts, theophylline, prostaglandins, and ATP. Mechanism and significance of this surprisingly sensitive response remain obscure.

INTRODUCTION

Although it is known that short chain fatty acids can be actively absorbed by small intestine [1–4], apparently nothing is known about possible effects of short chain fatty acids on the small intestine. In probing for such effects we discovered an electrical response to short chain fatty acids which seems intriguing because it can be elicited by surprisingly low concentrations.

METHODS

Adult male Sprague-Dawley rats, fed Purina laboratory chow and water ad libitum, were used. During anesthesia with pentobarbital, a 6–8 cm segment of either

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jejunum (beginning about 6–8 cm below the ligament of Treitz) or terminal ileum was cut at each end and rinsed with about 20 ml of Krebs-Henseleit solution [5] at pH 7.4 and 37 °C. Plastic cannulae were tied into each end of the segment; these were connected to the bottom of a reservoir drilled in a Lucite block which was positioned just above the animal. With the animal on its side, the cannulated segment with mesentery and blood supply intact was carefully placed in a plastic dish. Krebs-Henseleit solution gassed with 95 % O_2+5 % CO_2 was continuously dripped into the dish by gravity and the excess removed by vacuum. Krebs-Henseleit solution (8.0 ml) was also circulated through the lumen and reservoir by means of a bubble lift, gassing with 95 % O_2+5 % CO_2. The segment was positioned in the dish and the cannulae adjusted to assure adequate luminal and blood circulation. The entire experiment, including surgery, was performed in an environmental room at 37 °C.

Transmural electrical potential difference (PD) was measured from 3 M KCl agar bridges placed in the inside and outside solutions. The electrode tips were soaked in Krebs-Henseleit solution for at least 1 h prior to use. These bridges were connected to calomel reference electrodes and the potential difference was recorded on a Grass Model 5D polygraph through a Model 5P1 preamplifier. The potential due to electrode asymmetry was balanced out. We found that with the intestinal segment bathed outside the abdomen and the serosal electrode placed in the bathing solution, much more stable and reproducible recordings of transmural PD could be obtained than with the segment and serosal electrode placed in the abdominal cavity. However, the basic observations to be reported here were confirmed with the latter, more conventional, orientation, and also with the serosal electrode tied into the femoral vein.

After several minutes of equilibration and baseline recordings, 3.0 ml of the luminal solution were rapidly aspirated and immediately replaced with 3.0 ml of fresh Krebs-Henseleit solution containing enough test compound to achieve the desired concentration in the total luminal volume. The exchange was accomplished in about 3 s and exposure of the mucosal surface to the test compound began within the following 2 or 3 s.

Propionic, acetic, and formic acids as well as theophylline, 3′,-5′-cyclic adenosine monophosphate (cyclic AMP), dibutyryl cyclic AMP, and disodium adenosine 5′-triphosphate (ATP) were obtained from Sigma Chemical Company (St. Louis, Mo.). *N*-Butyric, valeric, hexanoic, and octanoic acids were obtained from Pfaltz and Bauer, Inc. (Flushing, N.Y.). Sodium taurocholate was obtained from Calbiochem (San Diego, Calif.). Prostaglandins E_1 and E_2 were kindly donated by the Upjohn Co. (Kalamazoo, Mich.). All short chain fatty acids were purchased as the sodium salts.

RESULTS

Effect of propionate

Control PD was about 3–4 mV, serosal side positive, and was remarkably stable for hours. Upon exposure of the jejunal mucosa to 10 mM sodium propionate a large increase in PD occurred with no measurable delay except that due to mixing time. PD reached a maximum value within 1 min, and then declined and reached exactly the control level in 3–5 min, at which it remained indefinitely. A sample

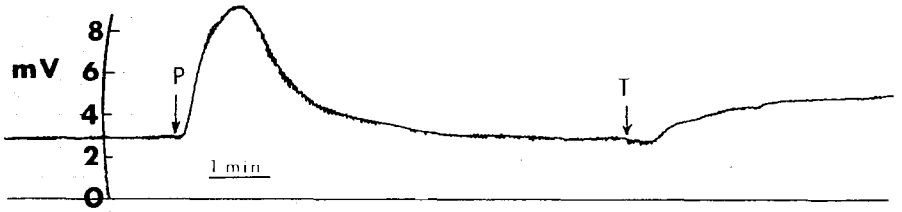

Fig. 1. Effects of 10 mM Na propionate and 10 mM theophylline on transmural PD in rat jejunum. PD is given in mV; serosal side is positive with respect to mucosal side. The vertical arrows indicate the times that propionate (P) or theophylline (T) was added to the mucosal reservoir. In this experiment considerable slow wave activity from smooth muscle was superimposed on transmural PD.

record is shown in Fig. 1. If the mucosa was exposed to 1.0 mM propionate and, following completion of the phasic response, another exchange was made which introduced an additional 1.0 mM propionate, no response resulted from the second addition. This result means that (1) the amplitude of the response is probably maximal at 1.0 mM, and (2) the declining phase is not caused simply by disappearance of propionate. On the other hand, if the lumen was rinsed with propionate-free medium after the phasic response was completed, a new response of normal amplitude could be elicited by a second addition of propionate. A sample experiment is shown in Fig. 2. This restoration of responsiveness could be repeated as many times as desired. It should also be noted from Fig. 2 that removing the propionate during the rinsing procedure had no effect on PD.

Fig. 3 shows the amplitude of the response in jejunum as a function of propionate concentration. A graded response was observed over a range from 1 to 50 μM. No further change in amplitude was observed from 50 to 1000 μM, indicating that the receptors involved in this response must have high affinity for propionate. At all concentrations tested the response was transitory and the PD returned precisely to its control value. The duration of the response tended to decrease as the concentration decreased.

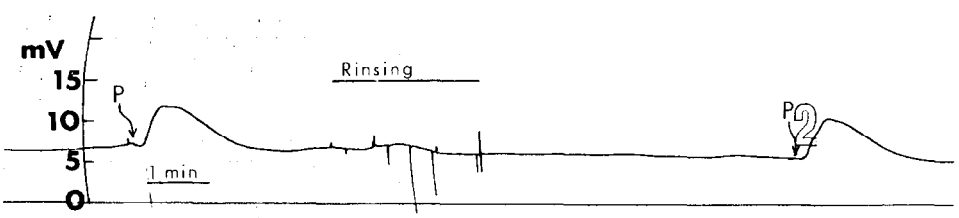

Fig. 2. This experiment illustrates that full responsiveness to propionate can be promptly restored merely by removing propionate from the lumen. The lumen was rinsed by repetitively aspirating the contents of the reservoir and injecting fresh propionate-free solution. During the rinsing procedure some electrical artifacts appear on the record. In this particular experiment the medium was simply 0.9 % NaCl gassed with O_2. The concentration of propionate (P) was 0.10 mM, but in other experiments recovery from refractoriness has been demonstrated when 10 mM propionate was used.

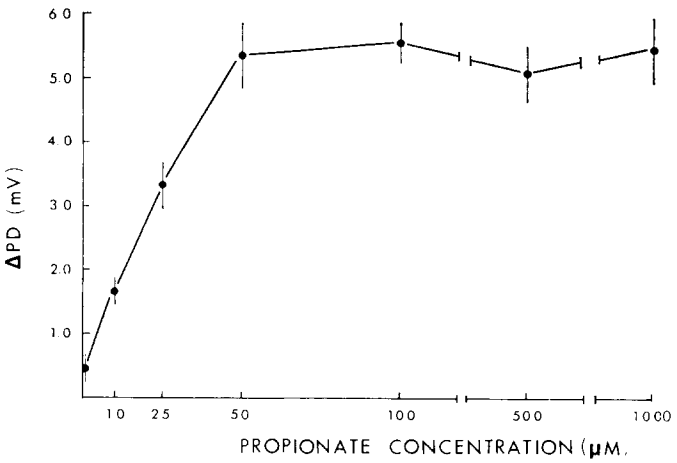

Fig. 3. Concentration dependence for the response to propionate. ΔPD is the amplitude of the response measured at its peak. Each point is the mean from 5 animals, except at 1.0 μM where 9 animals were used. Standard errors are indicated by vertical lines.

Effects of other short chain fatty acids

Several other short chain fatty acids ranging in chain length from C_1 to C_8 were tested at a concentration of 10 mM in segments of terminal ileum. Formate produced almost no response. The others all had effects very similar to that of propionate with respect to amplitude and time course. The sensitivity for each of these short chain fatty acids has not yet been determined, but we have found that either butyrate or valerate at 0.10 mM elicits nearly as large a response as that produced by 0.10 mM propionate.

We have also found that at various times during the response to 10 mM propionate, addition of a second short chain fatty acid has no detectable effect. Acetate, butyrate, and valerate were used in these experiments, each at a concentration of 10 mM. These results indicate that the various short chain fatty acids have a common effect.

Independence of this response from electrical responses to other materials

A number of other organic chemicals are known to induce an increase in transmural PD when applied to the mucosal surface of the gut, and we sought to determine if the response to short chain fatty acids was independent of these other responses. The responses studied were those to glucose [6], glycine and alanine [7], taurocholate [8, 9], theophylline [10], prostaglandins E_1 and E_2 [11], and ATP [12, 13]. With each of these materials, a maximally effective concentration (determined in preliminary experiments) was introduced into the intestinal lumen, and then after the expected effect on PD had developed, propionate was introduced. Alternatively, in each case, a maximally effective concentration of propionate was introduced first, followed by the test material. In no case was the usual effect of propionate altered (increased or decreased) by the presence of one of these other materials, nor were the effects of these other materials altered by the presence of propionate. A few sample records are shown in Figs. 1 and 4. We conclude that the response to

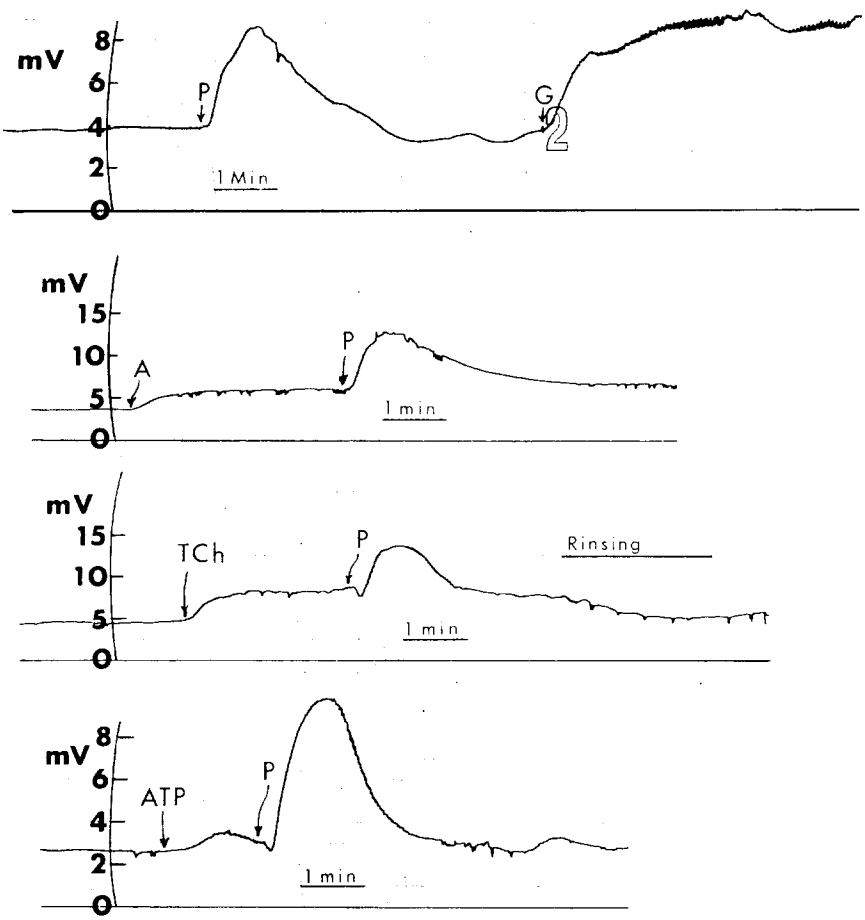

Fig. 4. Experiments which illustrate that the response to propionate is independent of the responses to a variety of other materials. P = propionate (1.0 mM except for top experiment in which propionate concentration was 0.10 mM). G = glucose (20 mM). A = alanine (20 mM). TCh = taurocholate (10 mM). ATP (1.0 mM). The two lower records were obtained from segments of terminal ileum, the two upper records from jejunum. The various quicker perturbations in the records represent electrical activity from smooth muscle.

propionate is independent of all these other responses and, therefore, represents a new and distinct phenomenon.

The following comments should be made concerning the results shown in Figs. 1 and 4: (1) The response to propionate is as prompt in onset and as rapid in development as is the well known response to glucose. (2) Alanine differs chemically from propionate only by an α-amino group, yet its electrical response has an altogether different shape and does not interfere with that of propionate. (3) The transmural electrical response to taurocholate in ileum under in vivo conditions has not been published before; it is a stable response, unlike the transitory effect seen

* The effects of prostaglandins E_1 and E_2 are not illustrated here, but at 0.1 mM the responses were maximal and looked similar to that of 10 mM theophylline shown in Fig. 1.

in vitro [8, 9]. (4) The electrical effects of theophylline and prostaglandins E_1 and E_2 on the gut* are thought to be mediated by cyclic AMP [10, 11]; therefore, it seems likely that the response to propionate is not mediated by cyclic AMP, a conclusion which is supported by the very prompt onset of the response to propionate compared to that of theophylline (Fig. 1). (5) The amplitude of our transitory response to ATP in rat ileum was much smaller than others have previously reported [12]. (6) In the terminal ileum the response to propionate invariably started with a small downward deflection lasting a few seconds which then gave way to the change in PD characteristic of the jejunum.

We should also note that the usual effect of propionate has been obtained in an atropinized rat (0.4 mg atropine per kg of rat injected intraperitoneally). Therefore, this response is apparently not related to the response to acetylcholine reported by Hardcastle and Eggenton [14].

Effects of cyclic AMP and dibutyryl cyclic AMP

No effect was found with concentrations of cyclic AMP up to 10 mM in the lumen. However, 10 mM dibutyryl cyclic AMP consistently produced a response identical in size, shape and timing to that produced by 1.0 mM propionate, with the exception that sometimes PD did not return completely to control level. Furthermore, no effect of dibutyryl cyclic AMP could be obtained if it was introduced into the lumen at various times during and following a maximal phasic response to propionate. These observations were puzzling until we realized that dibutyryl cyclic AMP might be contaminated by free butyrate. Accordingly, an ether extract of acidified dibutyryl cyclic AMP was analyzed by gas chromatography (15% SP-1220 column, ionization detector). It was found that the preparation of dibutyryl cyclic AMP used in this study was contaminated 1.3% by weight with free butyrate. Thus a 10 mM solution of dibutyryl cyclic AMP contained 0.66 mM butyrate, more than enough to explain the effect of "dibutyryl cyclic AMP".

DISCUSSION

Propionate and other short chain fatty acids, when applied to the mucosal surface of rat jejunum or ileum cause a prompt transitory increase in transmural PD. This seems to be a new phenomenon, distinct from the electrical responses to a variety of other organic molecules. In jejunum, a half-maximal response to propionate was obtained at a concentration of only about 25 μM. This extremely high sensitivity, together with the observed promptness of onset, and reversibility, preclude the possibility that this is an uninteresting osmotic or destructive effect. The fact that propionate has a nearly maximal effect at a concentration as low as 50 μM rules out the possibility that it acts by binding Ca^{2+} or Mg^{2+} in the luminal medium. The hypothesis that this effect is mediated by metabolism of short chain fatty acids appears unlikely because of its high sensitivity to low concentrations of propionate, its promptness of onset, and the fact that propionate is very poorly metabolized by rat intestine [2]. The polarity of the response (serosal side positive) is opposite to that expected for an electrogenic lumen-to-blood transport of propionate itself, or for a streaming potential osmotically induced by luminal propionate.

Propionate and other short chain fatty acids are actively transported by rat

jejunum [2], and this transport is a Na^+-dependent process [15]. In analogy with other Na^+-dependent transport processes for organic solutes it might be predicted that propionate would induce a "transfer potential". However, the published half-saturation concentration for propionate absorption from rat jejunum in vitro [2] is three orders of magnitude higher than the "half-saturation" concentration for the effect on transmural PD reported here. We are led to believe that this change in PD is not a transfer potential.

We will describe three possible types of explanations for the shape of the response: (1) The rising phase and the falling phase might be caused by two separate electrogenic processes which are turned on sequentially by propionate, and which act oppositely on transmural PD. (2) The effect of propionate might be on an electrically neutral ion transport process and the PD changes only while a new steady state within the epithelium is being reached. (3) Propionate might activate a single electrogenic mechanism (such as mucosal-to-serosal Na^+ transport, or serosal-to-mucosal Cl^- transport) and this activation is followed by an automatic time-dependent inactivation process which can only be reversed by removing the propionate.

We feel that the first of these explanations is essentially ruled out by the following observations: (1) the rising phase and the falling phase have the same concentration dependency (i.e., the response is always in the serosal-positive direction and is always transitory regardless of concentration); (2) there is no effect on PD of withdrawing the stimulus (i.e., propionate); and (3) the PD returns exactly to its prestimulus value while the stimulus remains in the lumen, rather than to some new steady value. We also think that the second explanation is highly doubtful because of observations 2 and 3 above. Therefore, as a working hypothesis we favor the idea that short chain fatty acids have a transient effect on some electrogenic ion pump.

Beyond this hypothesis our present data do not lead to a model to explain the effect. In vitro studies will be necessary. Such studies are now being attempted but, unfortunately, in vitro intestine has so far not responded to low concentrations of short chain fatty acids.

It should be noted that the concentration of propionate required to produce a near maximal effect on transmural PD (50 μM) is two orders of magnitude lower than the concentration of short chain fatty acids that has usually been found in small intestine [16–18] and one order of magnitude lower than that found by Chernov et al. [19] in human small intestine. Therefore, it would seem that the epithelium is constantly under the influence of short chain fatty acids. It remains to be seen if this influence plays any important role in epithelial function.

ACKNOWLEDGEMENTS

This work was supported in part by a research grant (AM-12985) from the National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Md. We thank Dr. George Cherayil, Department of Pathology, Medical College of Wisconsin, for doing the gas chromatography.

REFERENCES

1 Smyth, D. H. and Taylor, C. B. (1958) J. Physiol. 141, 73–80
2 Barry, R. J. C. and Smyth, D. H. (1960) J. Physiol. 152, 48–66
3 Barry, R. J. C., Jackson, M. J. and Smyth, D. H. (1966) J. Physiol. 182, 150–163
4 Jackson, M. J. (1968) Life Sciences 7, 517–523
5 Krebs, H. A. and Henseleit, K. (1932) Z. Physiol. Chem. 210, 33–66
6 Barry, R. J. C. (1967) Brit. Med. Bull. 23, 266–269
7 Kohn, P. G., Smyth, D. H. and Wright, E. M. (1968) J. Physiol. 196, 723–746
8 Wall, M. J. and Baker, R. D. (1972) Life Sciences 11, 375–386
9 Wall, M. J. and Baker, R. D. (1974) Am. J. Physiol. 227, 499–506
10 Field, M., Plotkin, G. R. and Silen, W. (1968) Nature 217, 469–471
11 Kimberg, D. V., Field, M., Johnson, J., Henderson, A. and Gershon, E. (1971) J. Clin. Invest. 50, 1218–1230
12 Kohn, P. G., Newey, H. and Smyth, D. H. (1970) J. Physiol. 208, 203–220
13 Gerencser, G. A. and Armstrong, W. McD. (1972) Biochim. Biophys. Acta 255, 663–674
14 Hardcastle, P. T. and Eggenton, J. (1973) Biochim. Biophys. Acta 298, 95–100
15 Jackson, M. J. and Smyth, D. H. (1968) Nature 219, 388–389
16 Dawson, A. M., Holdsworth, C. D. and Webb, J. (1964) Proc. Soc. Exp. Biol. Med. 117, 97–100
17 Argenzio, R. A., Southworth, M. and Stevens, C. E. (1974) Am. J. Physiol. 226, 1043–1050
18 Elsden, S. R., Hitchcock, M. W. S., Marshall, R. A. and Phillipson, A. T. (1946) J. Exp. Biol. 22, 191–202
19 Chernov, A. J., Doe, W. F. and Gompertz, D. (1972) Gut 13, 103–106