SUGAR-EVOKED POTENTIAL IN ISOLATED TOAD INTESTINE

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The transmural potential of the small intestine has been shown to be dependent on the presence of actively transported nonelectrolytes, such as D-glucose or L-alanine, in the fluid in contact with the mucosal surface.\(^1\)\(^-\)\(^4\)\(^,\)\(^9\)\(^,\)\(^14\) An addition of the actively transported sugar or amino acid into the mucosal fluid having contained no sugar or amino acid initially causes an immediate rapid increase of the mucosal negativity. Although the properties of this potential increment (the sugar- or the amino acid-evoked potential) have been studied in various animal species by different authors,\(^9\)\(^-\)\(^11\)\(^,\)\(^14\) the nature of its genesis has not yet been fully explained.

As shown by several authors,\(^9\)\(^-\)\(^11\)\(^,\)\(^14\) the sugar- or the amino acid-evoked potential is a phenomenon directly associated with the sodium-sugar or the sodium-amino acid interaction in the mucosal epithelial cells. Therefore, knowledge concerning its genesis seems to be of great importance for elucidating the sodium dependent active transport of nonelectrolytes by the intestine and some other tissues. This led us to an investigation on the nature of this potential. In our series of experiments, small intestines isolated from hibernating toads were employed because they exhibited quite stable sugar-evoked potentials. In the present study, the effects of different sugars on the transmural potential and the effects on the sugar-evoked potential of phlorizin and various external conditions were studied in order to see the characteristics of this particular potential exhibited by the toad intestine.

METHODS

Preparations: Hibernating toads (Bufo vulgaris) of either sex were used. The animals were anesthetized by intracisternal injection of 0.2-0.5 ml 25% urethane solution and almost the entire length of the small intestine was removed. The initial short segment of the duodenum above the opening of the common bile duct were excluded. The isolated intestine was washed with oxygenated Na-free (220 mM mannitol) buffer solution, then it was divided into six segments of nearly equal length, about 3 cm. Every seg-
SUGAR-EVOKED POTENTIAL IN TOAD INTESTINE

ment, numbered 1 to 6 starting from the duodenal end, was everted, and washed again with fresh Na-free buffer solution. Thereafter, the preparations were incubated in the same Na-free solution at room temperature (18–23°C) for about 30 to 120 min. The medium was aerated during the incubation. At the start of potential recording, one of the everted preparations was tied over a polyethylene cannula of 3-5 mm diameter and 5 cm length and the other end was ligated. The sac, thus suspended from a cannula, was filled with the same fluid as one to be used for the bathing solution of the outer surface of the sac. The fluid within the sac is referred to as the serosal fluid and the fluid outside the sac as the mucosal fluid. The latter was continuously aerated during experiments and its temperature was kept constant at 25°C. Na concentration of the fluid was varied in some experiments in which the effects of Na concentration were studied, but in most experiments, it was fixed at 17.4 mEq since this concentration was found to be sufficient to obtain nearly the maximum response. Moreover, relatively low Na concentration appeared to be effective in preventing the loss of reproducibility of the preparation during prolonged experiments. The desired Na concentration were obtained by mixing Na-free mannitol Ringer solution and Na₂SO₄-Ringer solution at various volume ratios. The composition of the former was as follows (in mM): mannitol 220, KHCO₃ 2.0, KH₂PO₄ 0.2, CaSO₄ 1.8, tris-buffer 5.0 (pH 7.8). The latter had the same composition as that of the former except for containing 87 mM Na₂SO₄ instead of 220 mM mannitol. The reasons for the use of SO₄⁻ as principal anions were to obtain a larger potential change than in Cl⁻ medium and to minimize the effect of fluid movement. In repeated experiments with a single preparation, the preparation and the bath were washed, after each test, with urea-Ringer solution containing no Na and sugar at least twice. The urea-Ringer solution contained 220 mM urea instead of 220 mM mannitol, otherwise it had the same composition as that of the mannitol-Ringer solution.

Recording of transmural potential: The potential difference across the wall of the sac was led out by connecting calomel half cells to the mucosal and serosal fluids by means of thin polyethylene tubes filled with 1 M KCl 2% agar and was recorded by use of a vibrating reed recorder, EPR-2TB, Towa Dempa Co. Ltd., Tokyo. The bridge asymmetry potential was checked before and after each test. The bridge system was renewed when its asymmetry potential became more than 1 mV.

Addition of sugars: After the spontaneous PD had attained a steady level, a certain small amount of nearly isotonic solution of one of sugars was rapidly injected into 20 ml mucosal fluid. The final concentration of the sugar applied was varied by changing the amount of the sugar solution to be added. Sugars examined were D-glucose, D-galactose, D-mannose, fructose and sucrose. In some experiments, two amino acids, glycine and α-amino isobutyric acid, were examined for the purpose of comparison. All these chemicals were supplied from Tokyo Kasei Co. Ltd., Tokyo. Phlorizin was supplied from Sigma Chemical Co., St. Louis.

RESULTS

Effects of various sugars. Segment 1 (the mid-duodenum) was used in most experiments since this portion exhibited much larger sugar-evoked potential than any other segments. The spontaneous PD in the absence of sugars was found to be dependent on Na concentration of the medium, and in a medium containing 17.4 mEq Na, the potential was either negative or positive on the
mucosal side with respect to the serosal side. The magnitude of the PD varied from $-5.8$ to $+7.5$ mV, the average value being slightly positive on the mucosal side, $+0.53\pm 4.1$ mV.

Five different sugars as listed above were tested. Each of them was added to the mucosal fluid to give the same final concentration of 5 mM. When D-glucose was added, an immediate rapid change in potential occurred. The potential change was an increment of the mucosal negativity when the mucosal side was negative before the addition of the sugar, while it was a decrease of the mucosal positivity or the reversal of polarity when the mucosal side was positive initially. In any case the direction of the potential shift was the same. The potential reached the maximum within 1.5-2 min, and the level thus attained was maintained constant at least for two hours. Actually, no time delay was seen between the time of sugar injection and the onset of the potential change when diffusion time required for sugar to access the mucosal surface was made minimum by a forced injection toward the mucosal surface.

The size of the evoked potential was dependent on various external conditions, such as Na concentration, sugar concentration, temperature and pH, but under certain fixed conditions, single preparations exhibited responses of nearly a constant size on repeated tests for a long period of time extending several hours. Such a long-lasting reproducibility was never seen during summer, when the preparations showed a rapid initial and subsequent steady time-dependent decline in the size of the evoked potential during repeated tests. The slope of the steady decline was 1.7 mV per hour on the average (6 cases).

D-galactose, which is known to be actively transported by the intestine of various animal species, had an effect quite similar to that of D-glucose. The size of the galactose-evoked potential, however, was distinctly smaller than that of the glucose-evoked potential when examined under the same conditions, the ratio of galactose- to glucose-evoked potentials being about 0.67. Successive addition of glucose after galactose resulted in an additional increment of potential size, but the sum of the potentials never exceeded the potential induced by glucose alone. No additional increment was seen when the sequence of addition of these two sugars was reversed (Fig. 1). This indicates that the preparation had greater affinity for glucose than for galactose.

Fructose and mannose, which are known to be metabolized, but not actively transported by the intestinal epithelial cells, produced no detectable potential change (Fig. 2). Successive addition of glucose after fructose or mannose produced a response of exactly the same magnitude as that induced by glucose alone. Sucrose, which is known to be split into monosaccharides at the digestive region of the brush border, produced a very small and slowly growing potential change in the same direction as that of the glucose-evoked potential.
FIG. 1. Effects of D-glucose and D-galactose on the transmural potential of isolated small intestine of toads. The sugars were added to the mucosal fluid at times as indicated by arrows. A bar shown on the bottom indicates 10 min interval.

FIG. 2. Effects of fructose, mannose and sucrose on the transmural potential of isolated toad intestine. A single preparation was used throughout experiments.
Effects of phlorizin. In the presence of $2 \times 10^{-4}$ M phlorizin the generation of the sugar-evoked potential was completely suppressed (Fig. 3). When added after glucose, phlorizin caused an immediate abolition of the glucose-evoked potential, so that the potential returned to the original level obtained prior to the addition of glucose. Phlorizin at this concentration had little effect on the spontaneous PD in the absence of glucose and on the glycine-evoked potential.

![Figure 3: Effects of phlorizin on the glucose-evoked potential. The glycine-evoked potential was recorded for comparative purpose. Upper small intestine (segment 1).](image)

Comparison among different segments. Marked difference was seen in the size of the response among the segments taken from various parts of the small intestine. Under conditions of 17.4 mEq Na, 25°C, pH 7.8 and 5 mM glucose,
the average size was $8.49 \pm 2.31 \text{mV}$ in segment 1, $2.27 \pm 1.52 \text{mV}$ in segment 2 and less than $1 \text{mV}$ in segments through 3 to 6. In contrast, the glycine-evoked potential had a distribution pattern quite different from that of the sugar-evoked potential (Fig. 4).

**Effects of sodium and glucose concentrations in the mucosal fluid.** In this series of experiments, Na concentration of the medium was varied from 2.2 to 87 mEq and the amount of glucose solution was widely changed so as to give a range of 0.2 to 5 mM in the final glucose concentration. The data were summarized in Fig. 5 and 6, in which the reciprocals of the glucose-evoked potential ($1/V_s$) were plotted against the reciprocals of the glucose concentrations ($1/\text{[glucose]}$) in Fig. 5 and against Na concentrations ($1/\text{[Na]}$) in Fig. 6 according to the Lineweaver-Burk's way of plotting. As clearly seen, the lines for $1/V_s$ versus $1/\text{[glucose]}$ at different Na concentrations are all straight-linear and their slopes were dependent on the Na concentration of the mucosal fluid. The lines crossed the ordinate at a common point. Similarly, the lines representing $1/V_s$ versus $1/\text{[Na]}$ at different glucose concentrations are all linear and crossed the ordinate at a common point. The slopes of the lines were dependent on the glucose concentration in the medium finally obtained. Thus the sugar-evoked potential exhibited the Michaelis-Menten like relationship with both Na and sugar concentrations. Na and sugar interacted with each other in such
FIG. 5. The relationship between the size of the glucose-evoked potential and the glucose concentration of the mucosal fluid at three different Na concentrations of the medium. Ordinate: reciprocals of the size of the glucose evoked potential in mV⁻¹, abscissa: reciprocals of the glucose concentrations of the mucosal fluid in mM⁻¹. Upper small intestine.

FIG. 6. The relationships between the size of the glucose-evoked potential and the Na concentration of the medium at three different mucosal glucose concentrations. Ordinate: reciprocals of the glucose-evoked potentials in mV⁻¹, abscissa: reciprocals of the Na concentrations of the medium in mEq⁻¹×10. Upper small intestine.
a way that an increase in one of concentrations of these two substances lowered the apparent Km value for the other. The apparent Km for glucose varied from 2.2 mM at 8.7 mEq Na to 0.51 mM at 38.4 mEq Na, whereas the Km for Na varied from 18.2 mEq at 0.4 mM glucose to 1.3 mEq at 5.0 mM glucose.

Effects of temperature and pH of the medium. Temperature of the bathing fluid was varied from 4 to 32°C and the sugar-evoked potential was recorded from a single preparation at different temperatures. Fig. 7 shows an example of the results of this type of experiment. The size of the glucose-evoked potential increased linearly as the temperature was raised. Estimated Q₁₀ was 2.8 between 10 and 20°C and 1.6 between 20 and 30°C.

pH of the medium was changed by adding tris-buffer of known pH values. Before and after each test, pH of the medium was checked by means of a pH glass electrode. The glucose-evoked potential linearly increased with the increase in pH ranging from 6.6 to 8.2 (Fig. 8).
DISCUSSION

The sugar-evoked potential observed in isolated toad intestines showed many similarities in properties to those seen in mammalian intestines.\textsuperscript{5,6} For examples, a) the evoked potential is produced only by actively transported sugars, b) the production of the potential is completely suppressed by $10^{-4}$M phlorizin which is known to inhibit the active sugar transport without affecting cellular metabolism, c) the size of the potential reveals a Michaelis-Menten like relationships with both Na and sugar concentrations of the medium, d) a very unique interaction is seen between Na and glucose as revealed by the modification of the apparent Km for glucose by changing the external Na concentration and \textit{vice versa}, and e) the potential shift occurs with a rapid time course immediately after an addition of sugar. These qualitative similarities suggest that a common mechanism is involved in the genesis of the evoked potential among various animal species.

There are some distinct quantitative differences between toad and mammalian intestines. One of these is that, in toads, the portion capable of producing large sugar-evoked potentials was limited to only the upper small intestine, whereas in rats such area distributed evenly throughout the entire small intestine.\textsuperscript{5} This difference may be due to some different distributions of density of the sugar transport systems in the mucosal surface. The fact that the glycine-evoked potential had a distribution pattern quite different from that of the sugar-evoked potential seems to support this interpretation.

Another difference is the relative affinities of the generating mechanism of the potential for sugars and Na. In toads, galactose always produced smaller potential changes than equimolar glucose, the average ratio of the galactose- to the glucose-evoked potentials being 0.67, while in rats the same was reported to be 1.3.\textsuperscript{5} The apparent Km values for glucose were 0.51 mM at
34.8 mEq Na and 1.0 mM at 17.4 mEq Na in toads, the values being much lower than the value of 15.2 mM at 24.0 mEq Na in rats. The apparent Km for Na in the present study was 1.3 mEq at 5 mM glucose, whereas that for Na in rats estimated from Fig. 13 in Lyon and Crane's paper is approximately 100 mEq at 5 mM glucose. Smith showed that, in goldfish intestines, the evoked potential decreased when Na concentration in the mucosal fluid was lowered below 50 mM and vanished at about 15 mM. Thus, the upper intestine of toads has extremely high affinities for both Na and glucose in comparison with other animals.

It should be noticed that small intestines removed from hibernating toads showed the sugar-evoked potential with quite high stability and the production of the potential was reproducible for many hours when examined in a relatively low external Na concentration. In contrast, it is usually difficult to obtain such a long-lasting stability or reproducibility in mammalian intestines as pointed out by several authors and in summer toads, as described above. Smith demonstrated that in goldfish the glucose-evoked potential was reduced by previous acclimatization of the fish to a high temperature. No systematic study was made in the present study of the effect of the temperature acclimatization, but the preservation of summer toads in a refrigerator (5–10°C) for five to fifteen days was not enough to change the intestine of the animals into winter type. Although the nature of factors controlling the stability or reproducibility of the sugar-evoked potential is not clear, it could be said that the preparations described in this paper seem to be quite suitable for time-consuming quantitative experiments on the evoked potential.

The Q_{10} for the sugar-evoked potential obtained in the present study was comparable to that for the sugar absorption by frog intestine reported by Cordier et Worbe and to that for the glucose-evoked potential in goldfish intestine. On the other hand, Schultz and Zalsky obtained much higher Q_{10} (4.9) for their sugar-evoked increment of the short-circuit current in rabbit ileum. No information is available to discriminate whether the difference of Q_{10} between sugar-evoked increments of the transmural potential and of the short-circuit current is due to the difference in genesis of these two phenomena or simply due to the difference in animal species.

No systematic observation has been reported on the effect of pH on the sugar-evoked potential. In mammals, active sugar transport is reported to be maximum at pH 7.0, while, in amphibian intestines, the sugar transport is greater in alkaline and acid reaction than in neutral. In our study, the size of the glucose-evoked potential increased progressively as pH of the medium was increased from 6.6 to 8.2. Whether this pH dependency of the potential is consistent with that of sugar absorption is uncertain at present, and further studies would be needed in the same animal species and under similar conditions. Smyth and Wright demonstrated a pH dependency of the streaming
potential across the small intestine of rats. The relation of the potential to pH observed by them was of quite different nature from that of the sugar-evoked potential observed in the present study.

SUMMARY

1. The sugar-induced change in transmural potential was recorded \textit{in vitro} from everted small intestines taken from hibernating toads and the characteristics of the potential change were studied under various external conditions.

2. Qualitatively, the sugar-evoked potential of toad intestine had characteristics quite similar to those seen in mammalian intestine, and it was intimately related to the active sugar transport. The size of the glucose-evoked potential revealed a Michaelis-Menten like relationship with external Na and glucose concentrations, the apparent Km values for glucose and Na being dependent on the Na and glucose concentrations of the medium, respectively.

3. The portion of the intestine capable of producing a large sugar-evoked potential was confined to the upper small intestine, where the affinities for glucose and Na were remarkably high in comparison with intestines of other animals.

4. The size of the glucose-evoked potential linearly increased as temperature or pH of the medium was elevated in the temperature range of 4–32°C and in the pH range of 6.6–8.2.

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REFERENCES


