(0.5 mg/kg). Mean arterial blood pressure was monitored by a Statham pressure gauge. Heparinized plasma samples were deproteinized with perchloric acid and neutralized with 4 N potassium hydroxide. Supernates were counted in a Beckman scintillation counter. Diluted urine was counted directly in Aquasol (New England Nuclear, Boston, MA). Plasma counts were normalized by a factor based on infused radioactivity per kg dog weight.

*Results.* Figure 1 shows that after plasma AIB counts are reasonably constant following 60–90 min of infusion, the injection of endotoxin rapidly raises AIB levels for the duration of the experiment. This response is similar to the plasma alanine increase produced by *E. coli* endotoxin[5]. Figure 2 indicates that plasma AIB counts are higher at every sample interval when endotoxin is injected prior to the start of the AIB infusion.

Endotoxin consistently lowers arterial blood pressure so that renal filtration is curtailed. 3 control dogs with urethral catheters were infused with AIB for 30 min to assess the significance of this renal effect. An average of 2.7% (2.2, 2.3 and 3.5%) of the total infused AIB or about 117,000 cpm were recovered from the urine after several bladder washes. If total renal shutdown occurs in endotoxified dogs, adding these urine counts to an estimated 800 ml plasma and 1600 ml extracellular fluid (ECF) would raise control fluid (plasma plus ECF) only 49 cpm/ml at the 30-min interval. The difference between control and endotoxemic plasma at this sample time is 400 cpm/ml. Thus the maximum contribution urinary AIB can make to plasma counts is 12% of the observed difference and this assumes complete renal shutdown. Lymph collected from the thoracic duct of one dog approximated plasma counts as expected.

*Discussion.* Plasma levels of a metabolically inert amino acid infused into dogs are rapidly elevated after endotoxin administration. Plasma alanine has been reported to show a similar response to endotoxin[5]. The present findings suggest that nonmetabolic factors may be operating in the partitioning of both amino acids during endotoxemia. A renal factor seems to be minimal in the AIB experiments.

Membrane damage mediated by endotoxin does not appear to be involved in the AIB increase in view of the results in figure 2. In the first few min of infusion, tissue AIB pools are not in equilibrium with circulating plasma AIB. Even if endotoxin damaged cell membranes, not enough AIB would be present in tissue pools to escape into circulating fluids to raise plasma AIB counts to the extent shown in figure 2. This suggests that endotoxin blocks tissue uptake of AIB and/or causes major fluid shifts in the body.

1 This investigation was supported in part by the Office of Naval Research, Contract N0001476CO132. R.E.K., Postdoctoral Fellow, supported by HL07090. Address correspondence to J.J.S.
2 W.R. Beisel, Am. J. clin. Nutr. *30*, 1236 (1977).
3 R.W. Wannemacher, Am. J. clin. Nutr. *30*, 1269 (1977).
4 A.C. Groves, L.I. Wolf, P.J. O'Regan, C. Beach, C. Hasinoff and W.H. Sutherland, Surgery *76*, 533 (1974).
5 R.E. Kuttner and J.J. Spitzer, J. surg. Res. *25*, 166 (1978).
6 M.W. Noall, T.R. Riggs, L.M. Walker and H.N. Christensen, Science *126*, 1002 (1957).
7 D.L. Oxender and H.N. Christensen, J. biol. Chem. *238*, 3686 (1963).
8 R. Kuttner, J. Sims and M.W. Gordon, J. Neurochem. *6*, 311 (1961).

## Studies on the transmembrane ion currents in the smooth-muscle cells of the gastric fundus

V. Bury and K. Boev[1]

*Institute of Physiology, Ukrainian Academy of Sciences, Kiev (USSR), 23 April 1979*

*Summary.* Under voltage-clamp conditions fast $Ca^{2+}$-inward and early $K^+$-outward currents were recorded from the smooth-muscle cells of the gastric fundus. It is assumed that the less electrical excitability of these cells is due to the early activation of the outward current.

The smooth muscles of the fundic region of the stomach do not[2] or rarely[3-5] manifest spontaneous electrical and contractile activity. Electrical stimulation does not always lead to the occurrence of regenerative spike potentials[6-11]. Thus some authors[6] suggest the presence of electrically less excitable muscle fibres which determine the blockade or the inhibition of the propagation of excitation in the fundic smooth muscles. Using the voltage clamp method we studied the membrane ion currents with the purpose of elucidating the causes of the slight electrical excitability of the fundic smooth-muscle cells.

*Materials and methods.* Circular muscle strips, 0.2–0.3 mm wide and 10–12 mm long, were removed from the fundus of the guinea-pig stomach. The strips were kept in Krebs solution for 1–2 h and were then placed in a chamber with a double sucrose gap, whose test compartment was 0.4 mm wide. The experiments were carried out under current clamp and voltage clamp conditions[7] in normal Krebs solution at 36 °C. The Na-free solutions contained choline chloride instead of Na in the presence of atropine ($10^{-6}$ M). In some of the experiments $Ba^{2+}$ (2.5 mM) was substituted for $Ca^{2+}$.

*Results and discussion.* Polarizing currents applied under current clamp conditions led to the occurrence of electrotonic potentials in the fundic smooth-muscle cells. In some of the preparations a low depolarization was superimposed on the electrotonic potentials resembling local excitation which did not develop into an action potential even at high intensity of depolarizing current (figure 1, a). The current-voltage relation was linear as upon depolarization and the membrane resistance decreased with increasing current intensity (figure 1, c). The time constant of the membrane was 150–180 msec.

Under voltage-clamp conditions we observed a fast inward current which reached its maximum values within 5–10 msec. The development of the fast inward current was interrupted by the early activation of the fast outward current (figure 1, b). The dynamics of the ion currents through the membrane of the fundic muscle cells resembled the dynamics of development of the ion currents from the ureter[8] where the activation of the fast K-outward current was slightly delayed after the activation of the fast inward current. That is why the inward and outward currents overlapped in a wide time interval. This led to a considerable decrease of the resultant transmembrane currents. Unlike the ureter in the fundic muscle cells the fast outward current was greater and in some of the strips it reached such high values that it inhibited the inward

current completely (usually in the muscle cells of the upper part of the fundus). The current-voltage relation of these preparations did not show a region with negative resistance. In the strips removed from different parts of the fundus we observed different relationships between the fast inward and the fast outward current. In the muscle strips cut out nearer the gastric corpus the inward current was not completely inhibited. The current-voltage relation of these cells showed a region with negative resistance.

In Na-free solution the inward current amplitude did not change. In Ca-free solution no inward current was observed. The outward current remained the same (figure 2).

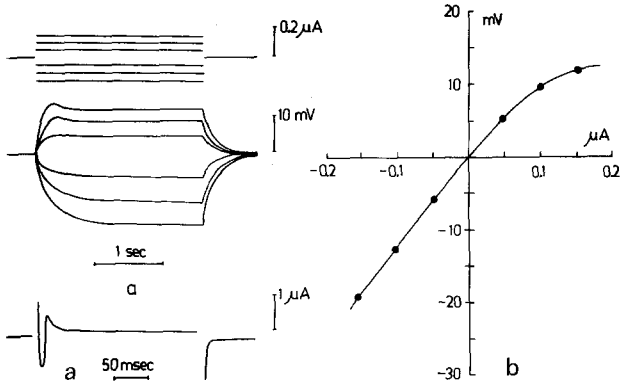


Fig. 1. *a* Responses of smooth-muscle cells from the fundic region of guinea-pig stomach to hyper- and depolarizing currents of different intensity. *b* Transmembrane current due to to step voltage change of 20 mV from the resting potential level. *c* Current-voltage relation of the membrane.

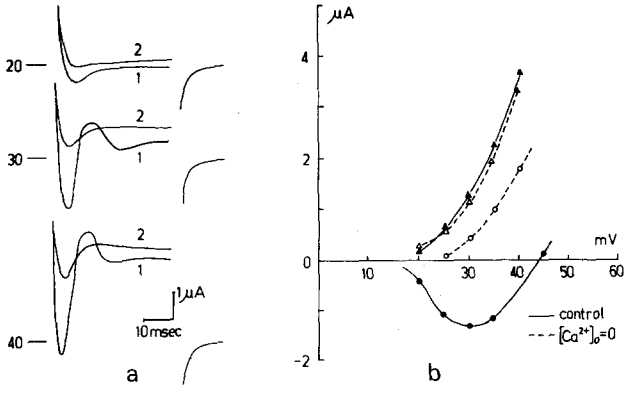


Fig. 2. Effect of Ca-free solution on transmembrane currents (a) and current-voltage relations ôB) for fast inward (●) and fast outward (△) current. 1 and 2, transmembrane currents in normal and Ca-free solution, respectively.

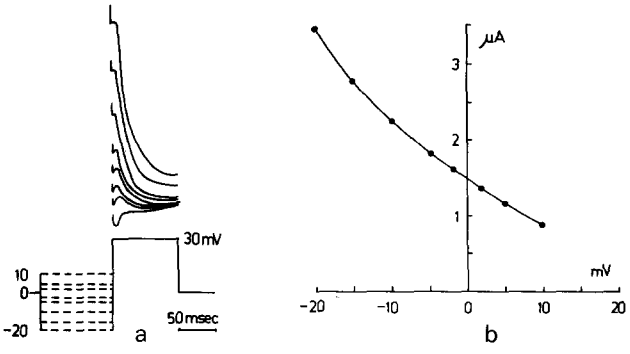


Fig. 3. Effect of conditioning hyper- and depolarization on the fast outward current.

$D_{600}$ ($10^{-5}$ M) and $Mn^{2+}$ ($5 \cdot 10^{-3}$ M) inhibited completely the inward current without changing the amplitude and the processes of inactivation of the outward current.

The amplitude of the fast outward current depended heavily on the magnitude of the holding potential. Figure 3 shows records of the fast outward current generated upon depolarization shifts of the membrane potential (+30 mV) at different levels of the conditioning potential (+10 to −20 mV) and duration of 100 msec. In order to avoid the influence of the inward current on the development of the outward current the experiments were carried out in the presence of $D_{600}$ ($10^{-5}$ M). From figure 3 it is seen that the outward current amplitude depends almost linearly on the magnitude of the conditioning potential.

Upon conditioning hyperpolarization the outward current was found to increase. This suggests that at the resting potential most of the channels of the fast outward current are in an inactivated state and the conditioning hyperpolarization removes this inactivation. Nevertheless, the non-inactivated channels of the fast outward current at resting potential are sufficient to inhibit the development of action potentials in the fundic smooth-muscle cells.

This suggestion is favored by the finding that the increase of the inward current upon membrane depolarization is accompanied by almost the same increase of the fast outward current. The charges carried through the membrane of both inward and outward currents are almost the same in the region where the current-voltage relation shows a negative resistance. As the processes of activation and inactivation of the transmembrane currents are completed within 15–20 msec the total charge carried by these currents for a time commensurable with the time constant will be nearly zero. Probably this is the fundamental cause for the absence of regenerative processes leading to the appearance of action potentials in the fundic smooth-muscle cells.

Under these conditions regenerative processes might develop if the balance between the fast inward and the fast outward current shifts towards the inward current. Experimentally this could be achieved by increasing the inward current through substitution of extracellular $Ca^{2+}$ by $Ba^{2+}$. As the Ca-channels are much more permeable to $Ba^{2+}$ than to $Ca^{2+}$ the substitution results in generation of high-amplitude action potentials of the type 'all-or-none' in all strips studied. When $Ca^{2+}$ is entirely replaced by $Ba^{2+}$ the inward current increases dozens of times and its reversal potential shifts towards the potential by 12 mV. Many authors explain the Ba-effect by a decrease of the conductance of the K-channels[10]. It was difficult in our experiments to evaluate the alteration of the fast outward current on the background of the sharply increased inward current under the effect of $Ba^{2+}$. However, bearing in mind the very small alteration of the reversal potential we could not rule out the participation of the fast outward current in the transmembrane currents.

The balance between the inward and the outward current in the fundic cells can shift towards the inward current when the outward current is blocked by tetraethylamonium (TEA). This might explain the appearance of spontaneous and evoked action potentials in muscle strips from the fundus of guinea-pig[6] and cat[11] stomach under the effect of TEA.

The results of the present study have shown that the absence of electrical excitability of the fundic muscle cells results from the early activation of the channels determining the fast outward K-current. The functional state of these channels might be changed by the effect of drugs and ions as well as by alterations in the magnitude of the cell membrane potential. Probably both mechanisms take part in the regulation of the electrical excitability of the fundic cells under normal conditions.

1 Present address: Institute of Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev St., B. 1, 1113 Sofia, Bulgaria.
2 T.Y. El-Sharkawy, K.G. Morgan and J.H. Szurzewski, J. Physiol. *279*, 291 (1978).
3 K. Boev, Proc. int. Union physiol. Sci. Paris XIII, 83 (1977).
4 M. Papasova, Rendiconti *3*, 137 (1971).
5 K. Golenhofen, D. v. Loh and K. Milenov, Pflügers Arch. ges. Physiol. *315*, 336 (1970).
6 T. Osa and H. Kuriyama, Jap. J. Physiol. *20*, 626 (1970).
7 V.A. Bury, Fiziol. Zh. SSSR *10*, 1608 (1973) (in Russian).
8 V.A. Bury, in: Physiology and Pharmacology of Smooth Muscle, p. 32. Ed. M. Papasova and E. Atanassova. Bulgarian Academy of Sciences, Sofia 1974.
9 S. Hagiwara, J. Fukuda and D.C. Eaton, J. gen. Physiol. *63*, 564 (1974).
10 Y. Sakamoto, Jap. J. Physiol. *20*, 610 (1970).
11 K. Boev, C.r. Acad. bulg. sci., in press (1980).

## Digestive enzymes in the gut and salivary gland of the larvae of *Chilo auricilius* Ddgn.

K. Somadder and M. Shrivastava[1]

*Indian Institute of Sugarcane Research, Lucknow 226 002 (India), and Department of Zoology, University of Lucknow, Lucknow 226 007 (India), 20 February 1979*

*Summary.* Amylase, α- and β-glucosidase, α- and β-galactosidase, β-fructosidase, trypsin, aminotripeptidase, leucine-aminopeptidase, prolinase, prolidase glycyl-L-leucine dipeptidase and glycylglycine dipeptidase are present in the 3rd instar larvae of *Chilo auricilius.*

*Chilo auricilius,* stalk borer, is a destructive pest of sugarcane in subtropical India. Sugarcane cultivars exhibit varying degree of resistance to this pest. Precise information on the basis of resistance, apparently connected with feeding behaviour of the pest, is not available. Artificial diets have been formulated for rearing this insect[2], however, and there appears to be scope for its modification so as to simulate the insect's behaviour and fecundity as found in nature.

Table 1. Carbohydrases in the gut and salivary glands of *Chilo auricilius* Ddgn.

| Enzymes | Salivary gland | Foregut | Midgut | Hindgut |
|---|---|---|---|---|
| Amylase | + | − | + | − |
| Cellulase | − | − | − | − |
| α-Glucosidases | | | | |
| a) Maltase | + | + | + | + |
| b) Sucrase | + | + | + | + |
| c) Trehalase | + | + | + | + |
| d) Melezitase | + | − | + | + |
| β-Glucosidase | − | − | + | − |
| α-Galactosidase | − | − | + | − |
| β-Galactosidase | − | − | + | − |
| β-Fructosidase | + | ± | + | + |

±, Slight activity; +, activity present; −, activity absent.

Table 2. Proteases in the gut and salivary glands of *Chilo auricilius* Ddgn.

| Enzymes | Salivary gland | Foregut | Midgut | Hindgut |
|---|---|---|---|---|
| Trypsin | − | − | + | + |
| Peptidases | | | | |
| Carboxypolypeptidase | − | − | − | − |
| Aminopeptidases | | | | |
| 1. Aminotripeptidase | + | + | + | + |
| 2. Leucine aminopeptidase | + | + | + | + |
| Dipeptidases | | | | |
| Prolinase | + | + | + | + |
| Prolidase | ± | − | ± | − |
| Glycyl-L-leucine dipeptidase | + | + | + | + |
| Glycyl glycine dipeptidase | + | + | + | + |

±, Slight activity; +, activity present; −, activity absent.

With this in view, work was initiated to determine the enzymes involved in the digestive system of the insect. The present report covers carbohydrases and proteases only in 3rd instar larvae.

*Materials and methods.* 3rd instar larvae of *chilo auricilius* were collected from the fields of sugarcane cultivar Co 1148 at the Institute farm in August–September. The larvae were immobilised by chilling at 0 °C for 10–15 min and dissected immediately after, in distilled water. Salivary glands and alimentary canal were taken out and collected in ice-cold distilled water. Fore-, mid- and hind-gut were collected separately and enzyme homogenates were prepared[3]. 16 larvae were used for 1 test. Enzymes were detected by paper chromatography[4]. The experiment was replicated 4 times.

*Results and discussion.* The enzymatic pattern of the larvae of *C. auricilius* has been elucidated in tables 1 and 2. The larvae secrete a wide range of carbohydrases, but they are not able to hydrolyse cellulose as cellulase is absent from their system (table 1). Amongst proteases tested, all the enzymes except carboxypolypeptidase are present in the digestive tract of the larvae (table 2). This indicates their ability to hydrolyse proteins besides carbohydrates.

*C. auricilius* is a pest of sugarcane, rich in sucrose. The larvae of this pest feed only on sugarcane almost all round the year. However, their wide enzymatic spectrum would enable them to digest a variety of proteins and carbohydrates. Hence the pest does not appear to be dependent upon sugarcane alone for its survival. It is well adapted to feed on alternate host plants under adverse conditions. However only 1 alternate host plant, *Sorghum helipens*[5], has been reported for *C. auricilius* so far.

The enzymatic pattern of the larvae is almost similar to that of the pink borer *Sesamia inferens*[3], and the leaf hopper *Pyrilla perpusilla*[6]. Both are pests of sugarcane, but have also been reported from a variety of other plants[7,8] with little sucrose. Unlike *C. auricilius,* both these pests are only active on sugarcane for a few months. There is a difference in their feeding behaviour as well; *S. inferens* usually feeds on sugarcane in the initial stages of the crop growth before sucrose accumulation starts, whereas *Pyrilla* feeds on leaves only. The main activity of the larvae of *C. auricilius* is in well developed stalks after sugar accumulation starts, but they are also found in young shoots.

1 Dr Kishan Singh, Indian Institute of Sugarcane Research, Lucknow, is gratefully acknowledged for providing necessary facilities.