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The influence of the γ-amino butyric acid (GABA) antagonist bicuculline on transport processes in rat small intestine

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Abstract—GABAergic involvement in the regulation of intestinal ion transport is suggested by the reduction in the short-circuit current generated by sheets of rat small intestine by the GABA antagonist bicuculline, although GABA itself caused no change. Bicuculline action was abolished by the inhibitor of Cl^- secretion frusemide, suggesting that it involved a change in endogenous Cl^- secretory tone. The effect of bicuculline appeared to be specific as it did not affect the electrical responses to glucose or exogenous secretagogues. The action of bicuculline was not observed in stripped intestinal sheets where the myenteric plexus is absent, and it was reduced by tetrodotoxin and atropine. It is suggested that endogenous GABA could be involved in the maintenance of a secretory tone in rat small intestine by acting on cholinergic mechanisms within the myenteric plexus.

γ-Amino butyric acid (GABA) is a neurotransmitter that has been identified in the myenteric plexus of the intestinal tract (Jessen 1981) where it is thought to play a role in the control of motility (Ong & Kerr 1982). As the enteric nervous system is also concerned with the regulation of the transport activity of the enterocytes that line the intestinal lumen (Lundgren 1988), the aim of the present study was to determine whether GABAergic mechanisms within the gut wall are involved in the control of intestinal transport by investigating the actions of GABA and its antagonist bicuculline on in-vitro preparations of rat small intestine.

Materials and methods

Chemicals. D-Glucose, mannitol, atropine sulphate, acetylcholine chloride (ACh) and DMSO were obtained from BDH Chemicals Ltd, Poole, UK; GABA, bicuculline, frusemide, 5-hydroxytryptamine creatinine sulphate (5-HT) and tetrodotoxin (TTX) from Sigma Chemical Co., St. Louis, MO, USA, and prostaglandin E_2 from Upjohn Co., Kalamazoo, MI, USA.

Animals. Experiments were carried out on male Wistar rats (230–250 g) obtained from the Sheffield Field Laboratories and allowed free access to food and water. They were anaesthetized with sodium pentobarbitone (60 mg kg^{-1} i.p.).

Measurement of transintestinal electrical activity. The potential difference (PD), short-circuit current (SCC) and tissue resistance (R) were measured in-vitro using paired sheets from the mid-region of the small intestine. In most experiments intact sheets were used, but in some the muscle layers, together with the myenteric plexus, were removed (stripped sheets). Each intestinal sheet was mounted in an Ussing chamber with an aperture of 1·925 cm^2 and incubated at 37°C in Krebs bicarbonate saline (Krebs & Henseleit 1932) gassed with 95% O_2/5% CO_2. The serosal solution contained 10 mM glucose and the mucosal solution 10 mM mannitol and each had a volume of 7 mL. The PD was measured using salt bridge electrodes connected via calomel half cells to a differential input electrometer. Current was applied across the tissue using Ag/AgCl electrodes which made contact with mucosal and serosal solutions via wide-bore salt bridges. When short-circuiting the tissue a correction was made for the resistance of the fluid as described by Field et al (1971). R was calculated from PD and SCC measurements using Ohm's law and the values for each tissue pair did not differ by more than 25%.

After mounting, tissues were allowed to stabilize for 10 min, and then readings were taken at 1 min intervals. In all cases 5 min basal readings were obtained before the addition of test agents. The response to an agent was taken as the difference between the SCC obtained in its presence and that immediately before its addition. A rise in SCC is indicated by a positive value and a fall by a negative value. All agents except glucose were added to the serosal solution. Bicuculline was dissolved in dimethylsulphoxide (DMSO):154 mM saline (1:1), frusemide was dissolved in DMSO and prostaglandin E_2 in ethanol: 0·2% Na_2CO_3 (1:9). For the other agents the vehicle was 154 mM NaCl.

Expression of results. Results are expressed as mean values ± s.e.m. of the number of observations indicated. Unless otherwise stated the data were obtained using intact sheets. Significance was assessed using Student's *t*-test, paired or unpaired as appropriate.

Results

Basal electrical activity. In intact sheets the basal PD was 4·9 ± 0·1 mV, the SCC was 100·1 ± 2·5 μA cm^{-2} and the R was 51·7 ± 1·5 ohm cm^2 (n = 140). Corresponding values for stripped sheets were 4·1 ± 0·4 mV, 99·3 ± 9·4 μA cm^{-2} and 41·3 ± 2·4 ohm cm^2 (n = 16).

Effect of GABA. GABA, at concentrations of 10^{-7} M (n = 4), 10^{-5} M (n = 4), 10^{-3} M (n = 4) and 10^{-2} M (n = 4), had no significant effect on the SCC ($P > 0·05$ in all cases). The viability of these tissues was confirmed by their response to glucose (10 mM) with a rise in SCC (72·2 ± 8·5 (16) μA cm^{-2}) that was not significantly different from control values (82·3 ± 7·3 (16) μA cm^{-2}, $P > 0·05$).

Effect of bicuculline. Bicuculline caused significant decreases in PD and SCC without affecting R (Fig. 1). At a concentration of 10^{-6} M it reduced the SCC by 10·8 ± 1·7(6) μA cm^{-2} ($P < 0·01$) and at 10^{-4} M by 23·8 ± 2·2(8) μA cm^{-2} ($P < 0·001$). The presence of bicuculline did not inhibit the response to subsequently administered glucose (10 mM) which increased the SCC by 110·0 ± 4·8(6) μA cm^{-2} with 10^{-6} M and 111·7 ± 8·4(8) μA cm^{-2} with 10^{-4} M. Corresponding control values were 110·8 ± 7·4(6) μA cm^{-2} and 92·2 ± 10·0(8) μA cm^{-2} ($P > 0·05$ in both cases).

Effect of bicuculline on stripped sheets. In contrast to its actions in intact sheets, bicuculline failed to cause a significant reduction of the SCC in stripped sheets, 10^{-4} M changing the SCC by only −3·3 ± 1·3(4) μA cm^{-2} ($P > 0·05$).

Effect of agents that influence neural activity on the response to bicuculline. The addition of TTX (10^{-5} M) to the serosal solution reduced the SCC by 30·7 ± 9·5(5) μA cm^{-2}. It also abolished the effect of 10^{-4} M bicuculline (control: −21·8 ± 4·5(5) μA cm^{-2};

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+TTX: $-2{\cdot}1 \pm 2{\cdot}5(5)$ μA cm^{-2}, $P < 0{\cdot}01$). This was not a non-specific inhibitory action as the response to PGE_2 (3×10^{-6} M) was not reduced (control: $57{\cdot}1 \pm 3{\cdot}3(5)$ μA cm^{-2}, +TTX: $56{\cdot}4 \pm 3{\cdot}7(5)$ μA cm^{-2}, $P > 0{\cdot}05$).

The muscarinic antagonist atropine (10^{-5} M in the serosal solution) reduced the SCC by $21{\cdot}6 \pm 2{\cdot}9(10)$ μA cm^{-2}. An effective inhibition of the cholinergic response was demonstrated by the reduction in the ACh (10^{-3} M)-induced rise in SCC from $70{\cdot}8 \pm 4{\cdot}4(4)$ to $13{\cdot}7 \pm 3{\cdot}9(4)$ μA cm^{-2} ($P < 0{\cdot}01$). The subsequent addition of bicuculline (10^{-4} M) caused a decrease in SCC of $9{\cdot}1 \pm 3{\cdot}0(10)$ μA cm^{-2} in the presence of atropine compared with a fall of $23{\cdot}4 \pm 2{\cdot}7(10)$ μA cm^{-2} ($P < 0{\cdot}01$) in its absence.

Effect of frusemide on the response to bicuculline. Frusemide (10^{-3} M) reduced the basal SCC by $12{\cdot}6 \pm 5{\cdot}2(6)$ μA cm^{-2} ($P < 0{\cdot}05$) and decreased the response of the intestine to PGE_2 (3×10^{-6} M) from $50{\cdot}3 \pm 3{\cdot}5(6)$ to $15{\cdot}6 \pm 3{\cdot}6(6)$ μA cm^{-2} ($P < 0{\cdot}01$). It also abolished the effect of 10^{-4} M bicuculline (control: $-20{\cdot}4 \pm 3{\cdot}7(6)$ μA cm^{-2}; +frusemide: $-2{\cdot}6 \pm 2{\cdot}6(6)$ μA cm^{-2}, $P < 0{\cdot}01$).

Effect of bicuculline on the electrical responses to other secretagogues. Bicuculline (10^{-4} M) did not affect the responses to 3×10^{-6} M PGE_2 (control: $52{\cdot}6 \pm 5{\cdot}0(4)$ μA cm^{-2}, + bicuculline: $53{\cdot}1 \pm 7{\cdot}6(4)$ μA cm^{-2}, $P > 0{\cdot}05$), 10^{-3} M ACh (control: $52{\cdot}6 \pm 5{\cdot}0(4)$ μA cm^{-2}; + bicuculline: $59{\cdot}1 \pm 7{\cdot}1$ μA cm^{-2}, $P > 0{\cdot}05$) or 3×10^{-5} M 5-HT (control: $52{\cdot}0 \pm 5{\cdot}6(4)$ μA cm^{-2}; + bicuculline: $42{\cdot}9 \pm 2{\cdot}5(4)$ μA cm^{-2}, $P > 0{\cdot}05$).

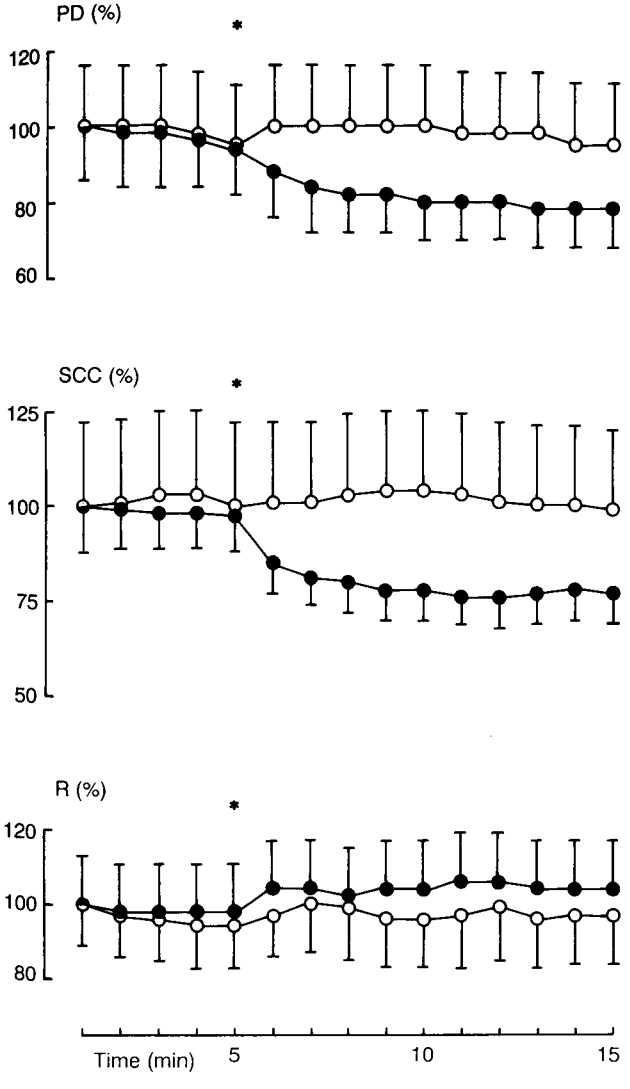

FIG. 1. Effect of bicuculline on the electrical activity of intact sheets of rat small intestine. Bicuculline (10^{-4} M) was added to the serosal solution of test sheets (●) at the time indicated by the star, while control sheets (○) received an equivalent volume of vehicle (1·4% v/v 50% DMSO: 50% 154 mM NaCl). The PD, SCC and R are expressed as % initial value and each point represents the mean ± s.e.m. of observations from 8 pairs of tissues. The initial PD was $4{\cdot}7 \pm 0{\cdot}5$ mV, the SCC was $84{\cdot}4 \pm 10{\cdot}0$ μA cm^{-2} and the R was $62{\cdot}2 \pm 5{\cdot}5$ ohm cm^2.

Discussion

The finding that bicuculline, an antagonist of GABA receptors, can alter the SCC generated by sheets of rat small intestine in-vitro suggests the involvement of GABAergic mechanisms in the control of intestinal ion transport. The action of bicuculline could not be attributed to a non-specific effect as the rise in SCC associated with Na^+-linked glucose absorption was not altered nor did the alkaloid affect the actions of secretagogues that act directly on the enterocyte. Previous in-vivo studies have demonstrated that intraperitoneal administration of the GABA agonist, muscimol, inhibits net water absorption while bicuculline causes a stimulation (Fogel et al 1985). These effects were attributed to a central action of the drugs as similar results were obtained when lower doses were administered into the cerebral ventricles. The present study however, indicates that there must also be a peripheral action, since bicuculline was able to influence ion transport in an isolated intestinal preparation (Fig. 1), although GABA itself had no effect. The failure to demonstrate a direct action of GABA could be attributed to the existence of endogenous GABA that may result in a maximal activation of GABAergic processes. Their inhibition by bicuculline reveals the involvement of such mechanisms in the regulation of intestinal transport.

There are currently considered to be at least two subtypes of GABAergic receptor—$GABA_A$ and $GABA_B$ (Eldefrawi & Eldefrawi 1987). Those authors reported bicuculline to be a competitive antagonist of the $GABA_A$ receptor which suggests that its effects on intestinal transport are mediated by this receptor subtype. This view is further strengthened by in-vivo studies in which muscimol, a $GABA_A$ agonist, reduced intestinal fluid absorption, while bicuculline enhanced absorption (Fogel et al 1985).

Although there is a recent report of the presence of GABA-immunoreactive cells in the rat intestinal epithelium (Davanger et al 1989) it is unlikely that bicuculline is acting at this level to influence ion transport. The site of bicuculline action is more likely to be the myenteric plexus as in the absence of this region of the enteric nervous system (stripped sheets), bicuculline failed to elicit a response. The neural origin of the decrease in SCC in intact sheets is demonstrated by its inhibition by TTX, a potent neurotoxin that blocks nerve conduction (Catterall 1980), and it is likely that a cholinergic mechanism is involved as it was also reduced by atropine. This is consistent with the actions of GABA on intestinal smooth muscle where it has been shown that the contraction mediated by $GABA_A$ receptors is mediated by the release of ACh (Krantis & Kerr 1981; Ong & Kerr 1982).

The action of bicuculline was reduced by frusemide, an agent that inhibits endogenous Cl^- secretion by blocking the Cl^- uptake mechanism at the basolateral membrane of the enterocyte (Frizzell et al 1979). Frusemide itself reduced the basal SCC which could be attributed to an inhibition of the endogenous Cl^- secretion that is known to occur in sheets of rat intestine (Munck 1970, 1972; Tai & Decker 1980; Hardcastle et al 1981a, b, 1984). This appears to be of neural origin as the basal SCC was inhibited by TTX and probably has a cholinergic component as atropine

also reduced the basal SCC. The results therefore suggest that the actions of GABA within the gut wall may contribute to the stimulation of endogenous Cl^- secretion, probably acting via the release of ACh. The observation that the intraperitoneal administration of a GABA agonist stimulates fluid secretion in-vivo, while an antagonist promotes absorption (Fogel et al 1985) is in accord with this view.

The finding that GABAergic agents can influence transport across the intestinal epithelium suggests that GABA should be added to the list of agents that may be involved in the neurohumoral regulation of the transport activity of the enterocyte.

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