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Alphaxalone reveals $GABA_A$ receptor-mediated inhibition in guinea-pig ileum

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Abstract—Contractions of the transmurally stimulated guinea-pig ileum were not affected by 10 μM bicuculline, a competitive $GABA_A$ receptor antagonist. The $GABA_A$ potentiator alphaxalone depressed the responses to 2 and 20 Hz stimulation by 20% and 40%, respectively, but had no effect on 0·2 Hz responses. The depressions were prevented by 10 μM bicuculline. This suggests that endogenous activation of $GABA_A$ receptors during transmural stimulation was normally subthreshold but, when pharmacologically potentiated, inhibitory effects were seen.

Neurons containing the amino acid γ-aminobutyric acid (GABA) are present in the myenteric plexus of the gut (Jessen et al 1979, 1987). There is evidence that both pre-loaded [^{3}H]GABA and endogenous GABA can be released upon electrical stimulation of isolated preparations of guinea-pig ileum (Taniyama et al 1983). Bicuculline-sensitive $GABA_A$ receptors coupled to chloride ion channels are present as, also, are $GABA_B$ receptors. Both types of receptor appear to be located entirely on myenteric neurones and not on the gut musculature (Krantis et al 1980). However, in the ileum, inhibitions by exogenous GABA of electrically stimulated contractions (Kaplita et al 1982) and stimulus-evoked excitatory post-synaptic potentials in myenteric neurones (Cherubini & North 1984) appear to be mediated solely via $GABA_B$ receptors.

In contrast, $GABA_A$ receptor activation has been found to have an excitatory effect on the ileum, although not in other segments of the gut. Exogenously applied GABA and other $GABA_A$ receptor agonists evoke the release of endogenous acetylcholine resulting in contraction of the ileum (Kaplita et al 1982; Kleinrok & Kilbinger 1983). A similar excitatory effect of endogenous GABA released by ethylenediamine and acting on $GABA_A$ receptors has been reported (Kerr & Ong 1984). It does not follow, however, that endogenous GABA would necessarily have an excitatory effect on cholinergic neurons that were already excited by parasympathetic activity. Such a situation can be mimicked by the simultaneous electrical stimulation of GABA-containing neurons and the parasympathetic innervation of the ileum. Thus, we have sought further evidence in the transmurally stimulated guinea-pig ileum by studying the effects of bicuculline and the $GABA_A$ potentiator alphaxalone (Harrison & Simmonds 1984).

Materials and methods

Segments of distal ileum, about 3 cm long, from the guinea-pig were set up for transmural stimulation and isometric recording of longitudinal contractions in Tyrode solution gassed with oxygen at 37°C. The resting tension was 2 g. Stimuli of 15 V, 0·2–0·5 ms wide, were delivered at frequencies of 0·2, 2 and 20 Hz for periods of 30 s every 3 min. The composition of the Tyrode solution was (mM): NaCl 149, KCl 2·7, $CaCl_2$ 3·6, $MgCl_2$ 2·1, NaH_2PO_4 0·4, $NaHCO_3$ 11·9, glucose 5·0.

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Bicuculline 10 μM and alphaxalone 0·01–1 μM were added to the organ bath at least 5 min before re-application of the sequence of electrical stimuli. Bicuculline (Sigma) was prepared as a 10 mM stock solution in 0·01 M HCl. Alphaxalone (Glaxo) was prepared as a 1 mM stock solution in ethanol. The maximum concentration of 0·1% ethanol that resulted upon dilution into the Tyrode solution has no effect on transmurally stimulated contractions of the guinea-pig ileum (M. Hawes & M.A. Simmonds, unpublished observations).

In some experiments, the effect of 0·1 μM alphaxolone was determined on contractile responses to 30 s applications of GABA.

Results

The peak tension developed in the ileum upon transmural stimulation increased with the frequency of stimulation. At 20 Hz, the initial peak tension was not fully sustained during the 30 s period of stimulation. In the presence of 10 μM bicuculline, the responses were unchanged (Fig. 1b).

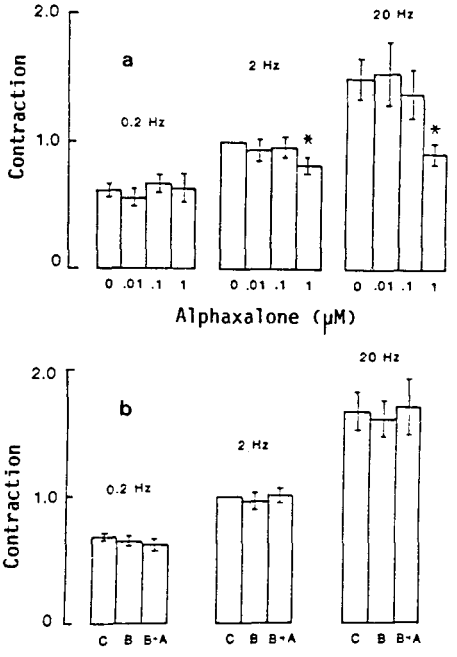

FIG. 1. Effects of alphaxalone and bicuculline on the isometric tension developed in guinea-pig ileum stimulated transmurally at 0·2, 2 and 20 Hz. Results from individual experiments were normalized with respect to the tension developed by 2 Hz stimulation which has a designated value of 1. The grouped data are shown as mean ± s.e.m. (a) Effects of cumulative concentrations of alphaxalone in 5 replicate experiments. (b) Effects of 10 μM bicuculline (B) in 7 experiments and 10 μM bicuculline + 1 μM alphaxalone (B + A) in 4 experiments compared with control responses (C) from 7 experiments. *Significantly different from 0 alphaxalone ($P < 0·05$, paired t-test).

To enhance the responsiveness of the $GABA_A$ receptor system to GABA, the ileum was exposed to cumulative concentrations of 0·01, 0·1 and 1 μM alphaxalone. The two lower concentrations had no effect but 1 μM alphaxalone decreased the responses to 2 Hz stimulation by 20% and the responses to 20 Hz stimulation by 40% (Fig. 1a). The latter responses also showed a more marked decline in tension following the peak. These effects of alphaxalone were prevented by 10 μM bicuculline (Fig. 1b).

Contractile responses to 100–200 μM GABA were elicited in 3 experiments and shown to be potentiated by 0·1 μM alphaxalone. Alphaxalone itself at this concentration had no direct effect on the resting tension and at 1 μM caused no more than a small transient increase.

Discussion

Bicuculline is a competitive $GABA_A$ receptor antagonist and the 10 μM concentration used in these experiments was about 10 times greater than its pA_2 concentration, as determined in a variety of tissues (see Simmonds 1986). The lack of effect of bicuculline on its own would suggest either that the responses to transmural stimulation were uninfluenced by endogenously activated $GABA_A$ receptors or that little $GABA_A$ receptor activation occurred. The latter possibility seems more likely since, in the presence of the selective $GABA_A$ potentiator alphaxalone, there was a $GABA_A$ receptor-mediated inhibitory effect at the two higher frequencies of transmural stimulation. These are the frequencies that have been shown to evoke the release of GABA (Taniyama et al 1983). It is unlikely that a direct GABA-mimetic effect of alphaxalone was involved since this should be independent of the frequency of stimulation.

The concentration of 1 μM alphaxalone found to be effective in the present experiment has previously been shown to be optimal for potentiation of the $GABA_A$ receptor agonist muscimol in the rat cuneate nucleus (Simmonds & Turner 1987). Lower concentrations of alphaxalone are able to potentiate contractile responses of the guinea-pig ileum to exogenous GABA (Ong et al 1988 and present results) although they did not affect responses to transmural stimulation. This may indicate that only very small amounts of endogenous GABA were released by transmural stimulation and that maximal potentiation by alphaxalone was required for such amounts to be suprathreshold.

By simple analogy with the effects of GABA on $GABA_A$ receptors in the central nervous system, it may be postulated that the inhibitory effect of endogenously released GABA in the guinea-pig ileum could result from reduced activation of excitatory neurones during transmural stimulation or a reduced release of transmitter from them. We have been unable to construct any feasible alternative explanation that assumes an excitatory effect of endogenously released GABA. There are, however, precedents for GABA being either inhibitory or excitatory under different circumstances in the same tissue. Thus, both exogenous and endogenously released GABA cause depolarizations of nerve endings in the central nervous system, although the underlying increase in chloride conductance normally has a dominant influence to result in presynaptic inhibition. Occasionally, however, the experimental conditions allow a transient excitation at the onset of GABA's action that results in the generation of action potentials and is the basis of the classical dorsal root reflex (see Levy 1977; Simmonds 1984). It is conceivable that something similar occurs when exogenous GABA releases acetylcholine in the ileum, particularly as the contractile response to GABA is not sustained in the continued presence of GABA (Ong et al 1988). However, at a time when cholinergic nerve endings are already being excited by electrical stimulation, it is not surprising that the simultaneous activation of $GABA_A$ receptors should have an inhibitory rather than an excitatory effect.

Thus, the nature of any physiological role of $GABA_A$ receptor activation in the ileum would depend upon the presence or absence of simultaneous parasympathetic nervous system activity. Whether a functional degree of $GABA_A$ receptor activation is achieved physiologically is, however, in question. In the transmurally stimulated guinea-pig ileum, we have been able to demonstrate such an effect only in the presence of a $GABA_A$ potentiator.

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