Baclofen: lack of effect on neurotransmission in the mouse vas deferens

R. H. EVANS, *Department of Pharmacology, Medical School, University Walk, Bristol BS8 1TD*

In order to define the selectivity in the depressant action of the antispastic drug baclofen it is necessary to test this compound on several different chemical neurotransmission systems. For instance transmission through the isolated superior cervical ganglion of the rat has been reported to be unaffected by levels of baclofen much higher than those required to depress transmission through the isolated spinal cord of the rat (Ault & Evans 1978). Also systemic baclofen has been shown to have no depressant action on cat Renshaw cells activated by ventral root stimulation (Kato et al 1978). Thus it would appear that both central and peripheral cholinergic transmission systems are resistant to this drug and its depressant action may be selective for a certain type of central excitatory synapse.

Baclofen is structurally related to GABA but the depressant action is unlikely to relate to this structural resemblance (Curtis et al 1974, Ault & Evans 1978). Curtis et al (1974) have suggested an alternative possibility, that the β-phenethylamine moiety of the molecule may be responsible for the depressant action. In this case baclofen might be expected to have some action on noradrenergic transmission.

The purpose of the present experiments was to observe the effect, if any, of baclofen on transmission in the mouse isolated vas deferens which contains a noradrenergic system (Hughes et al 1975). The action of baclofen was compared on transmission in the isolated hemisected spinal cord of the frog (Curtis et al 1961), transmission in the isolated superior cervical ganglion of the rat (Bowery & Brown 1974) and transmission in the isolated vas deferens of the mouse (Shaw & Turnbull 1978). The recordings shown in Fig. 1 are typical of observations made in at least three of each type of preparation.

In the frog spinal cord baclofen (50 μM) had a marked depressant action on the prolonged ventral root depolarization elicited by electrical stimulation of a corresponding dorsal root (Fig. 1a). The threshold concentration for a depressant action on the frog spinal cord preparation was 2 μM. Transmission in the superior cervical ganglion (Fig. 1b) and twitches of the mouse vas deferens elicited by electrical field stimulation (Fig. 1c) were unaffected by concentrations of baclofen up to 1 mM. However, metenkephalin at nanomolar concentrations depressed electrically evoked twitches in all four of the vas deferens preparations tested.

The depressant action of baclofen on transmission in

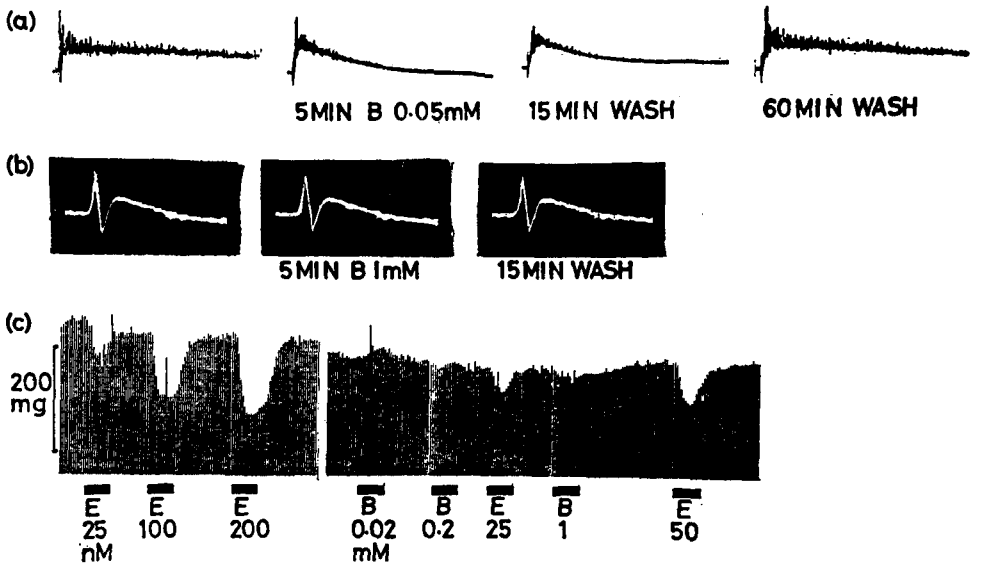

FIG. 1. (a) Isolated hemisected spinal cord of *R. temporaria* maintained at 10 °C. Oscilloscope recordings (d.c. coupled) of the ventral root depolarization elicited by electrical stimulation (supramaximal pulses, duration 0·2 ms, 1 min^{-1}) of a corresponding dorsal root. Calibration 4 mV, 400 ms.

(b) Isolated superior cervical ganglion of the rat maintained at 25 °C. Oscilloscope recordings (d.c. coupled) of the postganglionic response to electrical stimulation (supramaximal pulses, duration 0·5 ms 1 min^{-1}) of the preganglionic nerve. Each record consists of three superimposed traces. Calibration 4 mV, 40 ms.

(c) Isolated vas deferens of the mouse maintained at 32 °C, resting tension 100 mg. Isometric twitches elicited by field stimulation with 100 ms trains of 1 ms pulses (50 Hz) applied every 10 s at supramaximal intensity. Agonists applied during the period indicated by the bars below the record.

B = baclofen, concentrations mM, E = metenkephalin, concentrations nM.

the frog spinal cord is in agreement with the earlier observations of Davidoff & Sears (1974) who suggested that this compound has a selective blocking action at central excitatory synapses. Later results (Potashner 1978; Ault & Evans 1978) suggest that the depressant action is confined to terminals which release excitatory amino acids.

The present finding that transmission in the vas deferens is resistant to baclofen further emphasizes the selective action of this drug. It does not support the involvement of a β-phenethylamine receptor in this action (Curtis et al 1974) since β-phenethylamine, a sympathomimetic amine which acts indirectly by stimulating α-adrenoceptors in the mouse vas, would be expected to depress transmission in the vas deferens (Ambache et al 1972).

This work was supported by the Medical Research Council. Metenkephalin was provided by Wellcome Research Laboratories, Beckenham and baclofen was provided by CIBA-Geigy, Basle.

May 18, 1979

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Elimination of drugs by active intestinal transport

C. F. GEORGE*, BERYL S. GRUCHY, *Clinical Pharmacology Group, University of Southampton, Medical and Biological Sciences Building, Bassett Crescent East, Southampton S09 3TU, U.K.*

When drugs are recovered in the faeces after intravenous administration, it is often assumed that they have undergone excretion in the bile. However, with some drugs, metronidazole and ouabain for example, there is evidence that their removal in the faeces may be due, in part, to elimination across the intestinal wall (Ings et al 1975; Selden et al 1974). Acebutolol (DL-1-(2-acetyl-4-butyramidophenoxy)-2-hydroxy-3-isopropylamino propane hydrochloride), a β-adrenoceptor antagonist, is another drug which is handled in this manner. After intravenous administration to the dog, 35% can be recovered in the faeces and accumulation has been shown to occur in isolated sacs of canine intestine in vivo (Collins & George 1976). However, the mechanism of intestinal elimination of this (and other drugs) is not understood. We have, therefore, undertaken experiments both in vivo and in vitro in an attempt to elucidate this process.

The role of active and passive processes was initially determined by a study of the transport of [^{14}C]acebutolol (labelled on the ring carbonyl) against a concentration gradient in the dog. Three mongrel bitches (12–15·5 kg) were anaesthetized and intestinal loops measuring 15–17 cm were prepared in the manner described by George et al (1974). Each dog received an i.v. injection of [^{14}C]acebutolol 2–2·5 mg kg^{-1} (20–25 μ Ci) in 10 ml 0·9% w/v NaCl (saline) followed by a continuous infusion of half this amount per hour (since the half-life of the drug in this species is 1 h). Forty minutes after the bolus dose the loops were perfused for periods each lasting 10 min alternately with saline at 37 °C at a rate of 2·2 ml min^{-1} or 10^{-3} M acebutolol solution in saline for a total of 40 min. Blood samples were obtained from the aorta at 10 min intervals and radioactivity was measured by liquid scintillation counting. The nature of radioactive material in plasma was investigated by h.p.l.c. Perfusate from the loops was collected and the rates of elimination of [^{14}C]acebutolol were compared for the saline and acebutolol periods.

A further series of experiments was undertaken in vitro on segments of intestine from albino rats, ~250 g. Pairs of isolated everted intestinal sacs measuring 8 cm long were prepared from the upper 16 cm of the duodenum/jejunum. After adding [^{14}C]acebutolol solution (1 ml in Krebs) to the lumen these were incubated in Krebs bicarbonate solution and bubbled with 5% CO_2 in oxygen. One of each pair of sacs was treated with a possible inhibitor of active transport. In one series of experiments the temperature of the fluid surrounding the experimental loop was varied between 20 and 52 °C. In another, anoxia was produced by gassing with nitrogen: potential inhibitors which have been studied include cyanide 10^{-3} M, 2,4-dinitrophenol 10^{-4} M, ouabain 10^{-4} M, probenecid 10^{-3} M and ethacrynic acid 10^{-3} M. 3-*O*-Methylglucose and 2-deoxyglucose were tried as substitutes for glucose in the Krebs medium. Finally, the transport of acebutolol against a concentration gradient was studied at an initial concentration of 10^{-3} M acebutolol on both serosal and mucosal sides. Samples of incubate were removed at 15, 30, 45 and

* Correspondence.