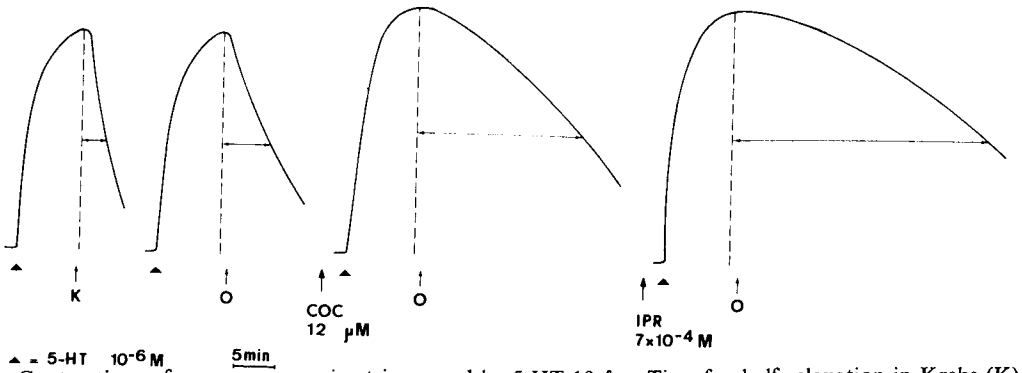


FIG. 1. Contractions of a saphenous vein strip caused by 5-HT $10^{-6}$ M. Time for half-relaxation in Krebs (K) and in oil (O) is represented by the horizontal arrows Iproniazid (IPR) slows the relaxation down much more than cocaine (COC); see Table 2.

with the reservation that in some cases (e.g. histamine) the concentrations needed to cause reasonable contractions may be too high and exceed the metabolizing capacity of the strip. It is clear that diffusion is the major mechanism for the termination of the action of histamine and prostaglandin $F_{2\alpha}$, whereas 5-HT, phenylephrine and dopamine (in decreasing order) are essentially taken up by adrenergic nerve terminals. Oxidative deamination is responsible for the final metabolic fate of 5-HT and phenylephrine, and hydrolysis by cholinesterase for acetylcholine. Extraneuronal uptake (and, eventually, subsequent metabolism) appears to play a significant role only for dopamine (and of low concentrations of phenylephrine).

August 1, 1979

## REFERENCES

Guimarães, S., Osswald, W. (1969) Eur. J. Pharmacol. 5: 133–140

Kalsner, S., Nickerson, M. (1968) J. Pharmacol. Exp. Ther. 163: 1–10

Osswald, W., Guimarães, S., Coimbra, A. (1971) Naunyn-Schmiedebergs Arch. Pharmakol. 269: 15–31

# Effects of urogastrone on mechanical activities of the stomach and intestine of guinea-pig

ISSEI TAKAYANAGI, *Department of Chemical Pharmacology, Toho University School of Pharmaceutical Sciences, Miyama, Funabashi, Chiba 274, Japan*

Urogastrone has been established as an inhibitor of gastric secretion (Lawrence et al 1971; Gregory 1970) and has been used clinically in gastric ulcer. However, the effects of urogastrone on the mechanical activity of smooth muscles remain obscure. Therefore I have examined the mode of action of urogastrone on gastrointestinal smooth muscle.

Female Hartley guinea-pigs, 300 to 400 g, were killed by a blow on the head, the ileum was isolated and a piece (3 to 4 cm) taken from the middle ileum was suspended in a 20 ml organ bath containing a physiological solution containing (g) NaCl 9·0, KCl 0·4, $CaCl_2$, 0·2, $MgCl_2$ 0·1, $NaHCO_3$ 1·0 and glucose 1·0 g in 1 litre) kept at 32 °C and gassed with 5% $CO_2$ in oxygen. Responses to drugs were recorded isotonically under a tension of 0·5 g. Drugs were added cumulatively to the bath fluid to obtain dose-response curves of the drugs in all experiments except those in Fig. 2. In some experiments electrical stimulation was according to Paton (1957). The electrodes were made of platinum and the intraluminal electrode was the anode. Rectangular pulses of 0·1 ms duration were used at frequencies of 0·1 and 50 Hz (for 2 s at intervals of 2 min) and strength sufficient to give a maximal response. The responses of ileum to electrical stimulation were recorded isometrically with an initial tension of 1·0 g.

The effect of urogastrone on the spontaneous movements of the stomach in situ was tested in the guinea-pig. A male guinea-pig (400 to 500 g) laparotominized under sodium pentobarbitone (30 mg $kg^{-1}$ i.p.) had a rubber microballoon implanted into the muscle layer of pyloric antrum. At least three days after the implantation and a fast of 24 h, the experiments were begun in the conscious animal. The internal pressure of the balloon was recorded by a low pressure transducer (Nagasawa et al 1974). Urogastrone used was kindly supplied from Tobishi Pharmaceutical Co. Ltd., Japan.

Dose response curves for acetylcholine, histamine and $BaCl_2$ were not influenced by urogastrone $3 \times 10^{-5}$ g $ml^{-1}$ but were slightly potentiated by $10^{-4}$ g $ml^{-1}$ (data not shown). The response of the ileum to electrical stimulation at 0·1 Hz was inhibited by urogastrone ($10^{-5}$ to

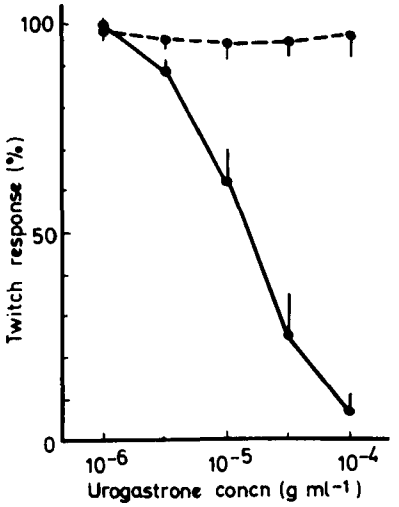


FIG. 1. Effects of urogastrone on the responses of guinea-pig ileum to electrical stimulation at 0·1 (solid line) and 50 (broken line) Hz. Values are presented as mean ± s.e. of 10 experiments.

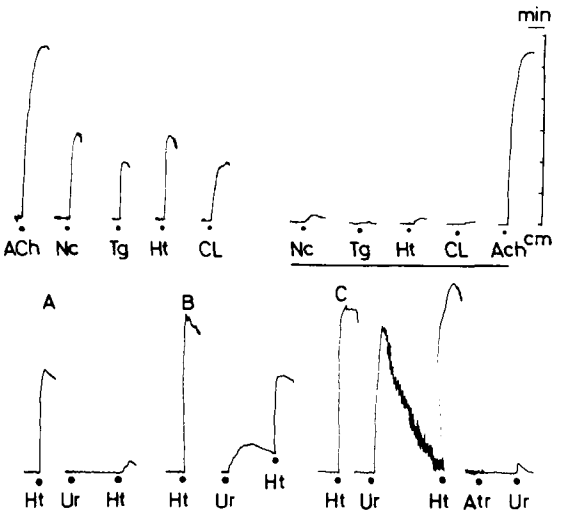


FIG. 2. Effects of urogastrone on the responses of guinea-pig ileum to some agonists. ACh: acetylcholine ($10^{-7}$ g ml$^{-1}$), Nc: nicotine ($10^{-6}$ g ml$^{-1}$), Tg: tetragastrin ($3 \times 10^{-6}$ g ml$^{-1}$), CL: caerulein ($10^{-9}$ g ml$^{-1}$), Ur: urogastrone ($3 \times 10^{-5}$ g ml$^{-1}$), Atr: atropine ($3 \times 10^{-7}$ g ml$^{-1}$). Horizontal bar: time scale, vertical bar: scale for contraction. Upper: inhibitory actions of urogastrone on the responses to nicotine, tetragastrin, 5-hydroxytryptamine and caerulein. Horizontal line: in the presence of urogastrone ($3 \times 10^{-5}$ g ml$^{-1}$). Lower: changes in effects of urogastrone on the response to 5-hydroxytryptamine after repeated dosing of urogastrone at $3 \times 10^{-5}$ g ml$^{-1}$. A, on the ileum treated with urogastrone once. B, on the ileum treated with urogastrone twice. C, on the ileum treated with urogastrone 6 times. These were observed on the same ileum.

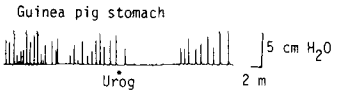


FIG. 3. Inhibitory effect of urogastrone (Urog; 60 mg kg$^{-1}$ i.p.) on the movement of the unanaesthetized guinea-pig stomach. Horizontal bar: time scale, vertical bar: scale for internal pressure of balloon.

$10^{-4}$ g ml$^{-1}$), but at the high dose of $3 \times 10^{-4}$ g ml$^{-1}$ it did not influence the response to electrical stimulation at 50 Hz for 2 s (Fig. 1). The finding that urogastrone inhibited the responses at 0·1 Hz but not those at 50 Hz are similar to those observed in experiments with morphine, strychnine, catecholamine and cAMP (Takagi & Takayanagi 1966, 1972; Paton & Vizi 1969; Greenberg et al 1970). The responses of the ileum to nicotine, tetragastrin, 5-hydroxytryptamine and caerulein, which were found to release acetylcholine from the cholinergic nerve in the ileum of guinea-pig, were much inhibited by $3 \times 10^{-5}$ g ml$^{-1}$ of urogastrone. In these experiments, the agonists were applied at intervals of 7 min and the ileum was pretreated with urogastrone for 3 min.

These results suggest that urogastrone decreases acetylcholine-release from the cholinergic nerve in the isolated ileum. When the ileum was repeatedly dosed with urogastrone at $3 \times 10^{-5}$ g ml$^{-1}$, some ileal segments (10 of 26 from different animals), showed tachyphylaxis, urogastrone's inhibitory action being decreased after 2 to 5 doses and disappearing after more than 5 doses. On the other hand, a stimulatory action of urogastrone was observed in preparations where its inhibitory action had decreased or disappeared. The stimulatory action was inhibited by atropine ($3 \times 10^{-7}$ g ml$^{-1}$) (Fig. 2).

Since spontaneous movement of the guinea-pig stomach in in situ experiments is completely inhibited by atropine, morphine and hexamethonium, the vagus nerve mainly controls movement of the stomach in situ (Terawaki et al 1976). Therefore, I have tested whether urogastrone inhibited the spontaneous movement of guinea-pig stomach in situ. Sixty mg kg$^{-1}$ (i.p.) of urogastone, the concentration necessary to inhibit acid output in the pylorus-ligated rat (T. Yamaura, personal communication), caused depression of spontaneous movement, which lasted 8 ± 3 min (mean ± s.e. of 8 experiments, Fig. 3). Saline was the vehicle. Movement of the stomach was not influenced by intraperitoneal injection of 0·4 ml of saline (a larger volume than that of the urogastrone solution). The stimulatory action of urogastrone was not observed in these experiments. My results suggest that urogastrone inhibits spontaneous movements of the gastrointestinal tract in situ through depression of acetylcholine-release from the cholinergic nerve and that this depression of acetylcholine-release is at least partly concerned with inhibition of gastric acid secretion by urogastrone.

In some ilea the mode of action of urogastrone was changed by repeated dosing ($3 \times 10^{-5}$ g ml$^{-1}$) this

might be the result of a change in the pharmacological properties of the nerve ending in the isolated preparations brought about by repeated doses.

September 17, 1979

REFERENCES

Greenberg, R., Kosterlitz, H. W., Waterfield, A. A. (1970) Br. J. Pharmacol. 40: 553P

Gregory, H. (1970) Digest. Dis. 15: 141–148

Lawrence, A. J., Schild, H. O., Smith, G. M. (1971) Br. J. Pharmacol. 41: 8–18

Nagasawa, K., Takayanagi, I., Miyamoto, M., Tomiyama, K., Takagi, K. (1974) J. Pharm. Pharmacol. 26: 833–835

Paton, W. D. M. (1957) Br. J. Pharmacol. 12: 119–127

Paton, W. D. M., Vizi, E. S. (1969) Ibid. 35: 10–28

Takagi, K., Takayanagi, I. (1966) Jpn. J. Pharmacol. 16: 211–216

Takagi, K., Takayanagi, I. (1972) Ibid. 22: 33–36

Terawaki, Y., Takayanagi, I., Takagi, K. (1976) Ibid. 26: 7–12.

# Baclofen: stereoselective inhibition of excitant amino acid release

G. A. R. JOHNSTON*, M. H. HAILSTONE, C. G. FREEMAN, *Department of Pharmacology, Australian National University, Canberra, Australia*

Baclofen, β-(p-chlorophenyl)-γ-aminobutyric acid (CIBA 34,647-Ba, Lioresal), is used clinically to reduce spasticity in various neurological disorders (Birkmayer 1972). Although first synthesized as an analogue of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) likely to penetrate the blood brain barrier, baclofen may inhibit the release of excitatory amino acid neurotransmitters at lower concentrations than those needed to influence GABA receptors or GABA release (Waddington & Cross 1979). Several studies have shown that baclofen may reduce primary afferent depolarization in the spinal cord by a depression of transmitter release (Davidoff & Sears 1974; Ault & Evans 1978; Curtis & Lodge 1978; Fox et al 1978; Kato et al 1978), and baclofen, 4 μM, has been shown to depress the electrically evoked release of endogenous glutamate and aspartate from slices of guinea-pig cerebral cortex (Potashner 1979).

The availability of the stereoisomers of baclofen, and the demonstration that the action on spinal reflexes resides with the (−)-isomer (Olpe et al 1978) has enabled the stereoselectivity of various actions of baclofen to be investigated. (−)-Baclofen is over twenty times more potent than the (+)-isomer in depressing synaptic activity in the immature rat isolated spinal cord (Ault & Evans 1978) and greater than two orders of magnitude more potent in its antinociceptive action in rats (Wilson & Yaksh 1978). On the other hand, potentiation of GABA release appears to be specific for the (+)-isomer (Kerwin & Pycock 1978), while both isomers are equally active in weakly inhibiting GABA receptor binding and in a GABA-dependent rotational behavioural test (Cross & Waddington 1978); these results provide further evidence against baclofen acting as a GABA-mimetic in its therapeutic effects.

The present investigations show that (−)-baclofen has a stereoselective action in inhibiting the evoked release of D-aspartate from slices of rat cerebral cortex and cat spinal cord. D-Aspartate is a good substrate for the high affinity acidic amino acid transport system that takes up the excitant amino acid neurotransmitters, L-glutamate and L-aspartate (Davies & Johnston 1976; Takagaki 1978). D-Aspartate appears to enter the presynaptic transmitter pools of L-glutamate and L-aspartate, and the uptake of D-aspartate has been used to study the localization of excitant amino acid-accumulating nerve terminals (Storm-Mathisen 1978). Preloaded D-aspartate can be released from these presynaptic pools in rat brain slices by increased potassium concentrations in a calcium-dependent manner, so that such evoked release of the metabolically stable D-aspartate may be a useful model for the synaptic release of the rapidly metabolized natural excitatory amino acid neurotransmitters (Davies & Johnston 1976).

The release of preloaded labelled D-aspartate and GABA from c.n.s. tissue slices was studied as described in detail previously (Davies et al 1975). The slices (0·1 × 0·1 × ca 2 mm) of rat cerebral cortex (removed after decapitation) or cat spinal cord (removed under

Table 1. Effects of baclofen stereoisomers on stimulated release of D-aspartate from c.n.s. tissue slices

| Conditions | Maximum efflux rate constant ($min^{-1}$) | % Control stimulated release |
|---|---|---|
| A. Slices of rat cerebral cortex: | | |
| (i) Potassium (40 mM) | | |
| Stimulation Control | 0·0055 ± 0·0003 (6) | 100 ± 5 |
| (−)-Baclofen 4 μM | 0·0039 ± 0·0004 (5) | 71 ± 7* |
| (+)-Baclofen 4 μM | 0·0060 ± 0·0007 (5) | 109 ± 13 |
| (ii) Protoveratrine (100 μM) | | |
| Stimulation Control | 0·0136 ± 0·0006 (10) | 100 ± 4 |
| (−)-Baclofen 1 μM | 0·0113 ± 0·0017 (3) | 83 ± 13 |
| 4 μM | 0·0073 ± 0·0009 (3) | 54 ± 7** |
| 10 μM | 0·0088 ± 0·0016 (3) | 65 ± 12* |
| (+)-Baclofen 1 μM | 0·0145 ± 0·0008 (3) | 107 ± 6 |
| 4 μM | 0·0121 ± 0·0008 (3) | 89 ± 6 |
| 10 μM | 0·0157 ± 0·0003 (3) | 115 ± 2* |
| B. Slices of cat spinal cord: Protoveratrine (100 μM) | | |
| Stimulation Control | 0·0097 ± 0·0005 (6) | 100 ± 5 |
| (−)-Baclofen 4 μM | 0·0055 ± 0·0004 (3) | 57 ± 4** |
| (+)-Baclofen 4 μM | 0·0099 ± 0·0006 (3) | 102 ± 6 |

Values are means ± s.e. of the number of determinations shown in brackets, and those labelled * and ** are significantly different from controls at $P < 0·02$ and 0·005 respectively by Student's *t*-test.

* Correspondence and present address: Dept. of Pharmacology, University of Sydney, N.S.W. 2006, Australia.