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The effect of reactive blue, an antagonist of ATP, on the isolated urinary bladders of guinea-pig and rat

L. K. Choo, *Department of Pharmacology, Victorian College of Pharmacy, Parkville, Victoria 3052. Australia*

Atropine resistance of the excitatory innervation of guinea-pig urinary bladder has been attributed to stimulation of purinergic nerves by Burnstock et al (1972, 1978a). However, Ambache & Zar (1970) failed to show a corresponding reduction of responses to nerve stimulation when tachyphylaxis to adenosine 5′-triphosphate (ATP) occurred. In the guinea-pig bladder it has been suggested that prostaglandins modulate the responses to the purine nucleotide (Burnstock et al 1978a). In accord with this suggestion, in the presence of indomethacin, reduction of responses to nerve stimulation occurred concomitantly with tachyphylaxis to ATP (Burnstock et al 1978a). Further studies supporting purinergic innervation in this tissue have been based on the similarity of responses to nerve stimulation and exogenous ATP in the presence of atropine. The demonstration of ATP release during nerve stimulation and quinacrine fluorescence indicating the presence of purinergic nerve terminals in the guinea-pig bladder also supported the purinergic theory (Burnstock et al 1978a,b).

The excitatory innervation of the rat bladder also exhibits atropine resistance (Carpenter 1963, 1977; Choo & Mitchelson 1977, 1980a) but is much less sensitive than the guinea-pig bladder to the purine, nucleotide, possibly because of increased metabolism (Brown et al 1979). In addition, the responses to nervous stimulation are reduced much less by prolonged contact with high doses of ATP in the presence of indomethacin plus hyoscine (Choo & Mitchelson 1980b).

Assessment of the role of purinergic nerve stimulation in atropine resistance has always been difficult because of a lack of a specific ATP receptor antagonist. Recently, an anthraquinone-sulphonic acid derivative (reactive blue 2) was reported to selectively antagonize the ATP-induced relaxation of guinea-pig isolated distal colon (Kerr & Krantis 1979). It was thus of interest to investigate the effect of this compound on the excitatory innervation of the guinea-pig and rat urinary bladders.

Urinary bladders isolated from Glaxo-Wistar rats (150–250 g) were set up for electrical stimulation according to Huković et al (1965). Detrusor strips were obtained from guinea-pigs (200–300 g) and their mucosa removed according to Ambache & Zar (1970). The tissue was placed under a resting tension of 0·5–1·0 g in a 10 ml organ bath in McEwen solution (McEwen 1956), maintained at 37 °C and gassed with carbogen. Responses were recorded on a Grass polygraph via a Grass force-displacement transducer (FT.03).

In electrically stimulated preparations, stimulation was performed over a frequency range of 1–50 Hz for 10 s at intervals of 2 min (rat) or 3 min (guinea-pig). In both preparations, responses were completely abolished by tetrodotoxin (0·31 μM). Dose cycles of 6 min with contact time of not more than 30 s, 60 s and 90–120 s were observed for ATP, acetylcholine (ACh) and potassium chloride (KCl), respectively.

Experiments on electrical stimulation were determined in the presence of guanethidine (3·4 μM), hyoscine (25 μM) and indomethacin (50 μM) to prevent the influences of adrenergic and cholinergic nerves and

Table 1. Effect of reactive blue on responses to electrical stimulation in guinea-pig isolated detrusor strip and isolated rat urinary bladder. Parameters of stimulation were: 2 ms pulse duration, using supramaximal voltage and frequencies ranging from 1 to 50 Hz. The preparation was stimulated for 10 s every 3 min (guinea-pig) or 10 s every 2 min (rat) in the presence of guanethidine (3 μM) hyoscine (25 μM) and indomethacin (50 μM) throughout.

Tissue	Reactive blue (μM)	Response* (% of control) Frequency (Hz) 1	2	5	10	20	50
Guinea-pig detrusor	20	65·9 ± 9·4 (4)	74·9 ± 13·0 (4)	57·6 ± 8·2 (4)	63·9 ± 5·6 (4)	54·4 ± 5·7 (4)	60·5 ± 1·2 (4)
	100	20·7 ± 7·6 (4)	20·4 ± 7·8 (4)	26·1 ± 4·2 (4)	25·8 ± 4·5 (4)	23·7 ± 3·7 (4)	27·8 ± 3·8 (4)
Rat bladder	100	79·9 ± 5·1 (6)	76·9 ± 1·8 (6)	72·2 ± 6·7 (6)	68·0 ± 7·0 (6)	71·5 ± 5·2 (6)	70·3 ± 4·7 (6)

* All responses were significantly different from control with $P < 0.05$ or lower.

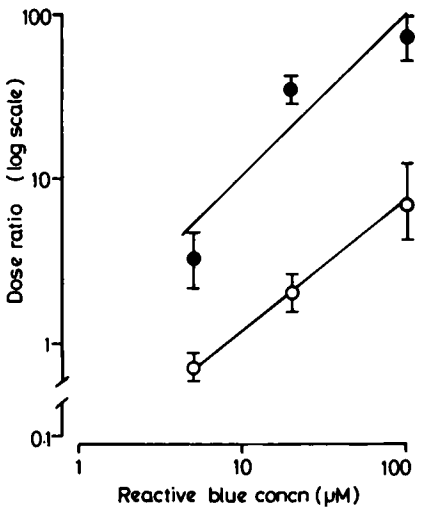

FIG. 1. Arunlakshana-Schild plots of the effect of reactive blue on responses to acetylcholine (○——○) (n = 4) in the presence of guanethidine (3·4 μM) and indomethacin (50 μM) and to adenosine 5′-triphosphate (●——●) (n = 6) in the presence of hyoscine (25 μM), guanethidine (3·4 μM) and indomethacin (50 μM) in guinea-pig detrusor strips. Each point is the geometric mean dose-ratio −1 (± s.e.m.).

the modulatory influences of prostaglandins. The tissue was incubated in the presence of these antagonists for 1 h before commencement of the experiment, the antagonists being replaced after each wash for the duration of the experiment. When control responses to either electrical stimulation or agonists had been established, reactive blue was added and allowed to equilibrate with the tissue for 1 h before further responses were established, the reactive blue being replaced following each wash.

Drugs used were: acetylcholine chloride (ACh), (Sigma); adenosine 5′-triphosphate (ATP), (Sigma); guanethidine sulphate (Ciba-Geigy); hyoscine hydrobromide (Drug Houses of Australia); indomethacin (Merck, Sharp & Dohme); potassium chloride (KCl), (Ajax); reactive blue 2 (Colour Index 61211), (Sigma). Student's *t*-test was used for all statistical comparisons.

In guinea-pig detrusor strips, reactive blue (5–100 μM) in the presence of guanethidine, hyoscine and indomethacin, was a potent antagonist of ATP, shifting the dose-response curve of the agonist rightwards, without affecting the dose-response curve to KCl (n = 4). The Arunlakshana-Schild plot (Arunlakshana & Schild 1959), using ATP as agonist, yielded a slope of 1·02 with a correlation coefficient of 0·94 (Fig. 1). The slope was not significantly different from 1, expected for competitive antagonism. However, in the presence of guanethidine and indomethacin, reactive blue also produced significant shifts in the dose-response curve to acetylcholine (Fig. 1). The Arunlakshana-Schild plot yielded a slope of 0·77 with a correlation coefficient of 1·0 which was significantly different from a slope of 1 ($P < 0.05$). The pA_2 values for reactive blue using ATP and ACh as agonists were 6·0 and 5·1 respectively.

Following pretreatment of the preparation with guanethidine, hyoscine and indomethacin, reactive blue (5–100 μM) also inhibited the responses to electrical stimulation over a range of frequencies (1–50 Hz) (Table 1). The inhibition produced at any one frequency was similar, there being no statistical difference ($p > 0.05$) between the degree of inhibition produced at 1 Hz and that obtained at 50 Hz.

In the rat isolated bladder preparation, in the presence of guanethidine, hyoscine and indomethacin, reactive blue (5–20 μM) produced no inhibition of the responses to ATP and only at 100 μM was a significant rightward shift in the dose-response curve to ATP observed (Fig. 2). The geometric mean dose ratio (95% confidence limits) from 4 experiments was 3·7 (1·4–9·9) (Fig. 2). At none of these concentrations were responses to KCl affected. In the presence of guanethidine and indomethacin, reactive blue (5–100 μM) had no effect on the dose-response curve to ACh. However, increasing the dose of reactive blue to 200 μM shifted the dose-response curve to ACh rightwards and a geometric mean dose-ratio of 3·5 (2·3–5·2:4) (95% confidence limits; number of experiments) was obtained.

Responses to electrical stimulation in the presence of guanethidine, hyoscine and indomethacin were inhibited significantly ($P < 0.05$ or lower) only with a high concentration of reactive blue (100 μM) (Table 1). The inhibition obtained with reactive blue (100 μM) at any one frequency was only 20–30% of the control response at the corresponding frequency, and there was no significant difference ($P > 0.05$) between the degree of inhibition obtained at 1 Hz to that obtained at 50 Hz.

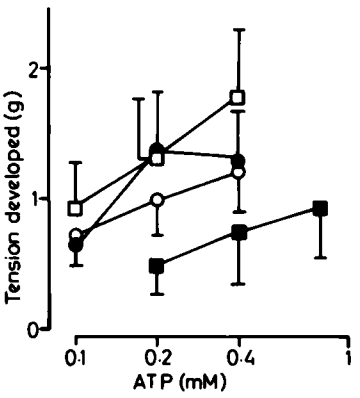

FIG. 2. Concentration-response curves to adenosine 5′-triphosphate (ATP) in the presence of guanethidine (3·4 μM), hyoscine (25 μM) and indomethacin (50 μM) in the rat urinary bladder in the absence of (○——○) and presence of reactive blue 5 μM (●——●), 20 μM (□——□) and 100 μM (■——■). Each point is the mean response from 4 preparations ± s.e.m.

Thus, although reactive blue was able to antagonize the responses to ATP in the guinea-pig detrusor, these concentrations of the antagonist also inhibited responses to ACh, although the latter appears to be inhibited in a non-competitive manner. It is not possible to equate the degree of blockade produced following electrical stimulation to the dose-ratio shifts obtained following agonist drug. However, the lack of complete inhibition of the responses to electrical stimulation of the guinea-pig detrusor strips (20–25% of maximal response still remaining) even at low frequencies of stimulation by a concentration of reactive blue (100 μM) capable of producing a 70 fold shift in the dose-response curve to ATP, would suggest that even if ATP is involved in the non-cholinergic, non-adrenergic excitatory response, it cannot be the only transmitter involved. One method of comparison would be to examine the effects of reactive blue on matching responses to electrical stimulation, ATP and ACh. However, this is not always possible, For example, in the rat bladder, where the sensitivity of the tissue to nerve stimulation and ACh is much greater that its sensitivity to ATP (Choo & Mitchelson 1980b), comparable responses cannot be obtained. In rat bladder, reactive blue (100 μM) showed only a very slight inhibitory effect against ATP and is thus not a useful tool for resolving the problem of whether ATP is involved in atropine resistance in the rat urinary bladder. Furthermore, the inhibition of responses to ACh by reactive blue also limits its usefulness in resolving whether the innervation of the bladder is entirely cholinergic as suggested by Carpenter 1977; Chesher 1970; Huković et al 1965; Ursillo & Clark 1956.

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Comparison of the effects of sodium salicylate with anti-ulcer agents in preventing indomethacin-induced intestinal ulcer

E. Ezer*, L. Szporny, *Pharmacological Laboratory, Chemical Works of G. Richter Ltd, H-1475 Budapest, Hungary*

In 1976, Ezer et al first described how the marked gastrointestinal ulcerogenic effect of indomethacin can be prevented by the simultaneous administration of sodium salicylate. The detailed results (Ezer et al 1979) were confirmed by others (Hayden et al 1978; Goburdhun et al 1978; Kyuki et al 1978; Rosenbaum et al 1979; Corell & Jensen 1979). The 1:10 combination of indomethacin with sodium salicylate (RGH-6705, Pelsonin) shows promising results clinically (Torgyán et al 1979).

Although the gastric ulcerogenic effect of non-steroidal anti-inflammatory drugs has been intensively studied the intestinal ulcerogenic effect has received much less attention possibly because the intestinal ulcers are more difficult to evaluate than gastric ulcers, and also because non-steroid inflammatory drugs bring about mainly gastric ulcers. Indomethacin, however, is an exception, it causes intestinal ulcers that can lead to peritonitis. We have examined the intestinal ulcerogenic effect of indomethacin and its prevention by sodium salicylate

Female Wistar rats, 120–150 g, *not* fasted before the treatments, were allowed free access to food and water during experiments. Under these conditions it requires at least 48 h for the intestinal ulcers to develop. To ensure the full expression of the ulcerogenic activity of indomethacin the animals were mostly killed 72 h after the drug was given. To evaluate the development of small intestinal ulcers, the tensile strength of intestinal wall was determined, by the inflation technique of Ezer et al (1976), because the erosion caused by ulcerogenesis leads to the weakening of the strength of the intestinal wall. The small intestine from pylorus to caecum was removed and the end was ligated, and a

* Correspondence.