J. Pharm. Pharmacol. 1990, 42: 597–598
Communicated March 26, 1989

Frusemide potentiates acetylcholine and carbachol in contracting the rat urinary bladder

J. N. OKPUKPARA, P. A. AKAH, *Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria*

Abstract—The interaction between acetylcholine and carbachol, and frusemide, a loop diuretic, have been studied on the rat isolated urinary bladder strip preparation. Acetylcholine ($4{\cdot}36\times10^{-8}$–$1{\cdot}3\times10^{-6}$ M) and carbachol ($5{\cdot}5\times10^{-8}$–$6{\cdot}9\times10^{-6}$ M) induced contractions and these were significantly potentiated by frusemide ($3{\cdot}02\times10^{-6}$ M). The ratio of EC50 in the absence of frusemide to EC50 in the presence of frusemide was $1{\cdot}58\pm0{\cdot}03$ (s.e.m.) for acetylcholine and $1{\cdot}86\pm0{\cdot}14$ for carbachol. Potentiation of acetylcholine and carbachol contractions by frusemide was not observed in tissues treated with hexamethonium ($2{\cdot}5\times10^{-5}$ M). Rhythmic contractions induced by frusemide alone were markedly reduced by hexamethonium ($2{\cdot}5\times10^{-5}$ M) and tetrodotoxin (10^{-6} M) but they were not significantly reduced by atropine ($1{\cdot}7\times10^{-6}$ M). The result suggests that frusemide increases the sensitivity of the bladder to acetylcholine and carbachol, and that it may have a nicotinic stimulant effect on the bladder. This extra-renal action may contribute to its prompt diuretic property.

Studies on the extra-renal effects of frusemide and related loop diuretics have shown that these drugs also (a) cause a slight decrease in bile flow (Erlinger et al 1970), (b) produce changes in ionic fluxes of isolated erythrocytes (Dunn 1973), (c) stimulate renin release from the kidney (Ganong 1972), and (d) augment urinary excretion of 5-hydroxytryptamine (Kassab et al 1973). The effect of frusemide on other parts of the urinary tract, e.g. urinary bladder, ureter and urethra, has not been fully investigated. As cholinomimetic stimulants, especially carbachol, are used in the treatment of bladder atony and urinary retention, the present study was designed to investigate the possible interaction between frusemide and cholinomimetic agonists on isolated strips of urinary bladder.

Methods

Adult albino rats of either sex, 180–200 g, were killed by a blow on the head and exsanguinated. The abdomen was opened and the urinary bladder rapidly excised above the bladder neck and freed of connective tissues. The bladder was opened by cutting along one side from the neck to the dome. The opened bladder was cut into three identical strips 3 to 4 mm wide and about 15 mm long. Each strip was suspended in a 10 mL organ bath containing Tyrode solution of composition (mmol L^{-1}): NaCl 137; KCl 2·7; $CaCl_2$ 1·0; NaH_2CO_3 11·9; NaH_2PO_4 0·2 and glucose 5·6. The temperature of the aerated solution was maintained at 37°C. The tissues were equilibrated for 60 min during which the bathing solution was replaced every 10 min. Contractions were recorded on a smoked paper attached to a rotating drum on a kymograph through an isotonic frontal writing lever with ×7 magnification. The resting tension on the tissue was 0·8 g. At the end of the equilibration period sequential responses were established for the agonists (acetylcholine and carbachol) acting for 60 s. The tissue was then incubated with frusemide for 30 min, after which responses to the agonists were re-established. Acetylcholine and carbachol were tested on different tissues. All responses to acetylcholine were obtained in the presence of physostigmine ($2{\cdot}5\times10^{-7}$ M). Time-matched control preparations were treated exactly like tested tissues except that they were not exposed to frusemide. The effect of hexamethonium ($2{\cdot}5\times10^{-5}$ M), tetrodotoxin (10^{-6} M) and atropine ($1{\cdot}7\times10^{-6}$ M) on frusemide-induced contractions were investigated. Additionally, the interaction between frusemide and the cholinomimetic agonists was examined in the presence of hexamethonium ($2{\cdot}5\times10^{-5}$ M). Results were expressed as means ± s.e.m. and where necessary mean values were compared using Student's *t*-test and the difference between means were regarded as significant when $P<0{\cdot}05$.

The drugs used were frusemide (Hoechst AG), physostigmine salicylate (Burroughs-Wellcome), acetylcholine chloride (BDH), carbachol chloride (BDH), atropine sulphate (BDH), hexamethonium (Koch-Light) and tetrodotoxin (Sankyo Co. Japan).

Results

Acetylcholine ($4{\cdot}36\times10^{-8}$–$1{\cdot}36\times10^{-6}$ M) and carbachol ($5{\cdot}5\times10^{-8}$–$6{\cdot}9\times10^{-6}$ M) caused concentration-dependent contraction of the rat urinary bladder strip. The −log EC50 values were $6{\cdot}86\pm1{\cdot}8$ and $6{\cdot}38\pm0{\cdot}17$ for acetylcholine and carbachol, respectively. Frusemide ($3{\cdot}02\times10^{-6}$ M) significantly ($P<0{\cdot}05$) enhanced these acetylcholine and carbachol-induced contractions (Fig. 1). Frusemide also increased the maximal responses to the agonists (Fig. 1). The ratio of EC50 in the absence of frusemide to the EC50 in the presence of frusemide was $1{\cdot}58\pm0{\cdot}03$ for acetylcholine and $1{\cdot}86\pm0{\cdot}14$ for carbachol.

In the presence of frusemide alone ($3{\cdot}02\times10^{-6}$ M), the

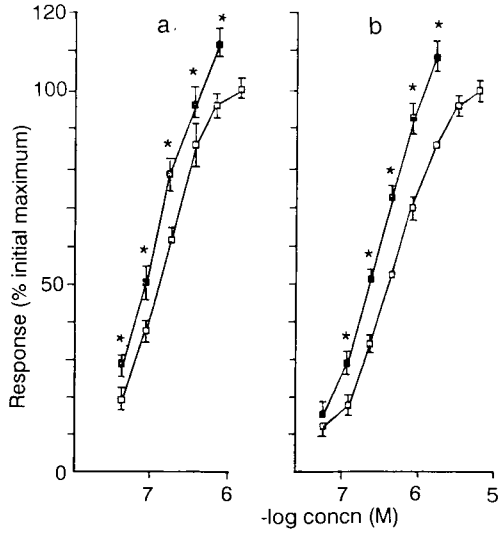

FIG. 1. Potentiation by frusemide ($3{\cdot}02\times10^{-6}$ M) of (a) acetylcholine and (b) carbachol in the rat urinary bladder strip: □——□ responses observed in the absence of frusemide; ■——■ responses observed in the presence of frusemide ($3{\cdot}02\times10^{-6}$ M). Each point is a mean ± s.e.m. of 5 observations from 5 experiments. * Indicates $P<0{\cdot}05$. In the case of experiments with acetylcholine, physostigmine ($2{\cdot}5\times10^{-7}$ M) was present throughout.

Correspondence to: P. A. Akah, Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria.

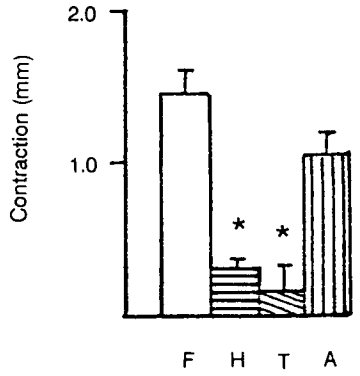

FIG. 2. Effect of hexamethonium ($2{\cdot}5 \times 10^{-5}$ M; H), tetrodotoxin (1×10^{-6} M; T) and atropine ($1{\cdot}7 \times 10^{-6}$ M; A) on frusemide ($3{\cdot}02 \times 10^{-6}$ M; F)-induced rhythmic contractions of the rat urinary bladder strip. Each bar is a mean ± s.e.m. of 5 observations from 5 experiments. * Indicates a significant difference from contractions observed in the presence of frusemide alone ($P < 0{\cdot}05$).

normally quiescent preparation developed regular and well-maintained rhythmic contractions. These contractions were markedly reduced by hexamethonium ($2{\cdot}5 \times 10^{-6}$ M) and tetrodotoxin (10^{-6} M), while atropine ($1{\cdot}7 \times 10^{-6}$ M) caused a small reduction in the amplitude of contractions (Fig. 2).

Finally, we looked at the responses to acetylcholine and carbachol (with and without frusemide) in the presence of hexamethonium ($2{\cdot}5 \times 10^{-6}$ M). In these experiments, hexamethonium (preincubation for 30 min) did not appear to change the concentration response curves to acetylcholine and carbachol (Fig. 3). The ratio of the EC50 in the absence of hexamethonium to the EC50 in the presence of hexamethonium was $0{\cdot}8 \pm 0{\cdot}45$ for acetylcholine and $1{\cdot}02 \pm 0{\cdot}14$ for carbachol. The frusemide-induced potentiation of acetylcholine and carbachol responses was abolished by hexamethonium (not shown).

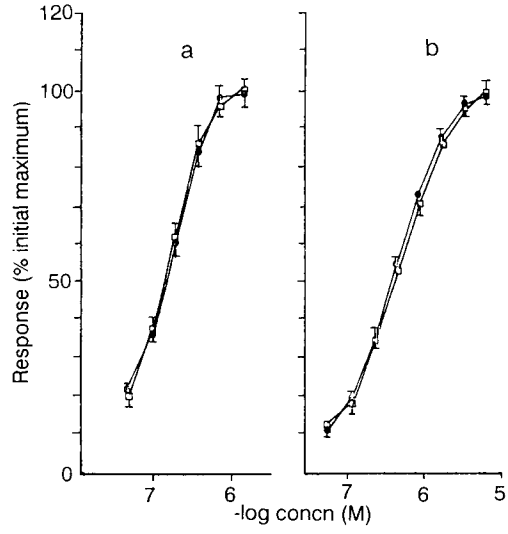

FIG. 3. Dose response curves of (a) acetylcholine, (b) carbachol without □——□ and with ●——● hexamethonium ($2{\cdot}5 \times 10^{-5}$ M). Each point is a mean ± s.e.m. of 5 observations from 5 experiments. In the case of experiments with acetylcholine, physostigmine ($2{\cdot}5 \times 10^{-7}$ M) was present.

Discussion

The results presented here show that frusemide enhanced the responses of the isolated rat urinary bladder strip to acetylcholine and carbachol. The inhibition by hexamethonium of the effects of frusemide suggests that frusemide can act on nicotinic cholinoceptors which could, for example, result in the release of mediators from the terminals of postganglionic nerves. This could also explain the development of spontaneous contractions by the preparations when frusemide was added. Another possibility is that frusemide stimulates the ganglia of the hypogastric nerves to the bladder. There is evidence that such stimulation causes contraction which is abolished by hexamethonium and potentiated by anticholinesterases (Mantegazza & Niamzada 1967). This suggests the release of an acetylcholine-like substance. We have no histological data, but Chesher's (1967) studies revealed that the rat urinary bladder is almost devoid of ganglion cells. Accordingly, it is doubtful whether the effect of frusemide is due to an action at parasympathetic ganglia or hypogastric ganglia. The effect of tetrodotoxin suggests that frusemide has an excitatory effect on the bladder strips by virtue of its ability to induce action potential discharge in neural elements within the bladder wall (assuming that tetrodotoxin does not have a direct effect on, for example, nicotinic receptors). This effect may not necessarily be localized to ganglion cell bodies, since nicotinic receptors are widely distributed in autonomic nerve fibres (Gyermek 1961). The relative ineffectiveness of atropine may indicate the relative importance of nicotinic receptors or even the release of stimulating substances other than acetylcholine, perhaps the activation of a non-cholinergic, non-adrenergic system. Atropine-resistant contraction of the urinary bladder has been documented (Hukovic et al 1965; Ambache & Zar 1970; Akah 1986). The mechanism by which frusemide potentiates the responses of these agonists on the bladder is not yet clear. The common side effects of frusemide therapy such as muscle cramps, urinary urgency and diarrhoea may be related to this effect. The enhanced response of the bladder to cholinomimetic stimulants and the increase in maximal responses could perhaps result from a frusemide-induced increase in sensitivity of the preparation to activity in cholinergic pathways.

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