

THE EFFECT OF ETHACRYNIC ACID ON THE GUINEA-PIG AND RAT ISOLATED VAS DEFERENS

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- 1 The effect of ethacrynic acid (EA) was studied on guinea-pig and rat vas deferens *in vitro*.
- 2 EA contracted the guinea-pig but not the rat vas deferens in a dose-dependent manner (50-800 $\mu\text{g/ml}$). Tyramine caused contraction in 10 out of 18 guinea-pig vas deferens; EA caused contraction in 17 of the preparations which did not respond to tyramine. Repeated doses of EA produced tachyphylaxis, but there was no cross tachyphylaxis to tyramine.
- 3 The contractions produced by EA were prevented by phentolamine or reserpine pretreatment and potentiated by cocaine. A low concentration of desipramine (3 ng/ml) potentiated and higher concentrations (0.6 and 3.0 $\mu\text{g/ml}$) inhibited the response of vas deferens to EA.
- 4 Hexamethonium (100 $\mu\text{g/ml}$) or atropine (0.1 $\mu\text{g/ml}$) did not inhibit the effect of EA, excluding the nicotinic and muscarinic receptors as the sites of action.
- 5 The effect of noradrenaline (NA) on the guinea-pig and rat vas deferens was enhanced by EA pretreatment, which may be due to inhibition of NA uptake.
- 6 It is concluded that EA releases NA from guinea-pig vas deferens. The mechanism of release seems to be different from that of tyramine.

Introduction

Ethacrynic acid (EA) is a potent diuretic agent (Beyer, Baer, Michaelson & Russo, 1965). Long term treatment (7 to 8 days) of rats with EA depletes heart muscle of catecholamine, but does not change the noradrenaline (NA) concentration in kidneys, adrenal glands, epididymal fat tissue and brain (Torsti, Vapaatalo & Neuvonen, 1968). A previous report from this laboratory suggested that EA releases catecholamines from guinea-pig isolated atria (Pousti, Zarrindast, Sadeghi & Khoyi, 1973).

The effects of EA on smooth muscle preparations have not been extensively investigated. Baggio & Poggioli (1969) reported that pretreatment of rabbits with EA potentiated responses of the isolated aorta to NA. It has also been reported that EA has a vasodilator action independent of its diuretic effect (Ogilvie & Ruedy, 1971) without involvement of α - or β -adrenoceptor, cholinergic or histamine receptor mechanisms (Ogilvie, 1971).

In the present work we studied the effect of EA on guinea-pig and rat vas deferens.

Methods

Male guinea-pigs (300-350 g) and rats (200-300 g) were stunned by a blow on the head and

exsanguinated. The vasa deferentia were immediately excised and dissected free of mesentery and vascular tissue. They were placed in Tyrode solution of the following composition (mM) NaCl 136.8, KCl 2.7, CaCl_2 1.8, MgCl_2 1.1, NaH_2PO_4 0.4, NaHCO_3 11.9 and glucose 5.5. The solution was aerated with oxygen and maintained at 37°C. Isometric contractile force was recorded with a photosensitive transducer and a PMP-4A Physiograph (Naco Biosystems). Resting tension was adjusted to 1 gram. After equilibration for 1 h, the muscle was contracted with NA (3 or 24 $\mu\text{g/ml}$, exposure time 45 s) before carrying out the experiments. An interval of 3 min was allowed between successive doses of NA, tyramine or acetylcholine. This interval was 10 min for EA. Exposure times for different drugs were: EA 3 min, hexamethonium 10 min, atropine 5 min, cocaine 5 min, desipramine (DMI) 5 or 20 min and phentolamine 5 minutes. The drugs used were: (–)-noradrenaline bitartrate, tyramine hydrochloride, ethacrynic acid (Merck Sharp & Dohme) cocaine hydrochloride (May & Baker), phentolamine mesylate (Ciba Geigy), reserpine (Ciba Geigy), desipramine hydrochloride (Ciba Geigy), atropine sulphate and acetylcholine hydrochloride.

The significance of the difference between means was evaluated with Student's *t* test. Paired

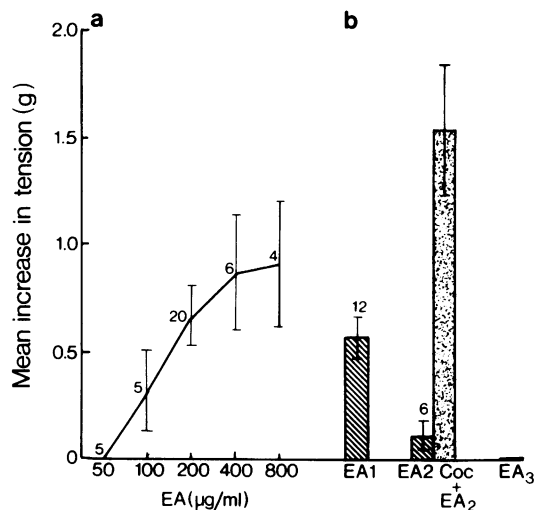


Fig. 1 Isometric contractions of guinea-pig vas deferens induced by ethacrynic acid (EA). (a) Responses to different doses of EA. In each preparation, only the response to the first dose of EA was considered. (b) The vasa were treated with EA (200 μg/ml) every 10 min (E_1 to E_3) until tachyphylaxis developed. After the first dose of EA half of the preparations were pretreated with cocaine (Coc, 1 μg/ml) before the second dose of EA was added. Figures indicate the number of experiments. Vertical lines show s.e. mean.

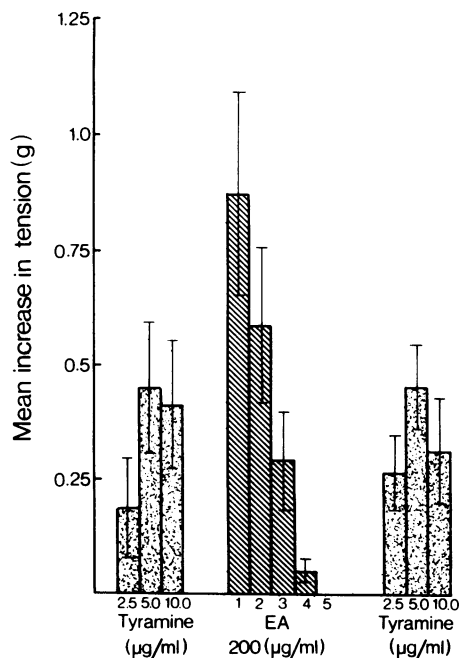


Fig. 2 Isometric contractions of guinea-pig vas deferens. Response to three doses of tyramine before and after the development of tachyphylaxis to ethacrynic acid (EA, 200 μg/ml). 10 experiments. Vertical lines indicate s.e. mean.

comparisons were used where they were appropriate for the statistical evaluation.

Results

Ethacrynic acid contracted the guinea-pig vas deferens. Tachyphylaxis developed during repeated applications. Therefore, in determining the dose-response relationship, only the response, to the first dose of EA was considered in each preparation. The results for different doses are shown in Figure 1a. The contractions appeared 1-2 min after drug addition, taking 0.5 to 3 min to develop fully. The lower the dose, the longer was the time to reach the height of contraction. Although there was much variation between different preparations, the mean contractile response showed dose-dependency. Concentrations of EA equal to or below 50 μg/ml had no effect, and maximal responses were obtained with 400-800 μg/ml. EA 400 μg/ml caused an increase in tension equal to that produced by 3 μg/ml of NA (Figure 3). The maximum tension developed in response to EA was higher than that to

tyramine but lower than that to NA (Figures 1, 2 & 4). There was no correlation between the magnitude of responses to EA and that to tyramine. In a group of 18 preparations, 8 vasa did not respond to tyramine (compare Ambache Dunk, Verney & Zar, 1972) while 17 contracted with the addition of EA (200 μg/ml). One vas deferens showed no response to EA but was contracted with tyramine.

In the study of tachyphylaxis to EA, a dose of 200 μg/ml, producing a submaximal response, was chosen in order to avoid agonist-induced desensitization which occurs in this preparation. The tissues were stimulated with EA every 10 minutes. The contractile response decreased with successive treatments (Figures 1 & 2). In preparations which contracted with tyramine, induction of tachyphylaxis to EA did not alter the magnitude of the response to tyramine (Fig. 2, 10 experiments).

Reserpine pretreatment (5 mg/kg, i.p.) 24 h before the experiment (6 guinea-pigs) inhibited the contractile response of vasa deferentia to EA ($P < 0.025$, Figure 3). The response to NA was slightly potentiated and tyramine (5 μg/ml) was without effect.

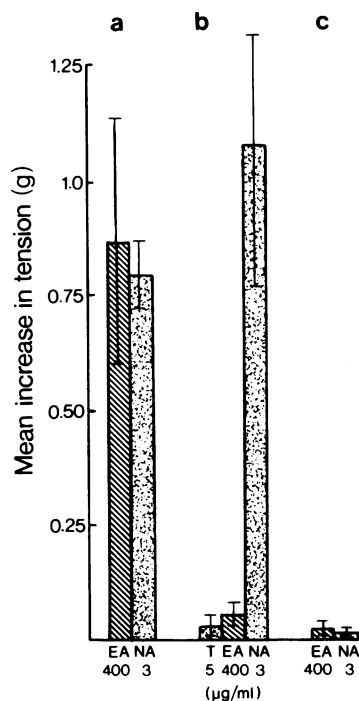


Fig. 3 Isometric contractions of guinea-pig vas deferens, induced by ethacrynic acid (EA, 400 µg/ml), noradrenaline (NA, 3 µg/ml) or tyramine (T, 5 µg/ml). (a) Control, (b) reserpine pretreated (5 mg/kg, 24 h before experiment), and (c) pretreated with phentolamine mesylate (1 µg/ml). Each point is the mean of 6 experiments. Vertical lines indicate s.e. mean.

The effect of an α -adrenoceptor blocking agent, phentolamine, on EA- and NA-induced responses was examined in 6 experiments (Figure 3). In the presence of phentolamine, 1 µg/ml, the contractile responses to EA (400 µg/ml) as well as to NA (3 µg/ml) were completely prevented ($P < 0.02$ and $P < 0.005$, respectively).

In 6 experiments, after the first dose of EA was washed out, cocaine, 1 µg/ml, was added to the bathing medium and left for 3 minutes. It did not cause any contractions. The subsequent contraction, induced by EA, was significantly greater than the response to the first dose of EA (Fig. 1b, $P < 0.005$).

In 15 guinea-pigs, the effect of desipramine hydrochloride (3 ng–3 µg) on the response of vasa deferentia to EA was investigated. One vas deferens of each animal was used as control for comparison. The results are shown in Figure 4b. Desipramine at concentrations of 0.6 and 3 µg/ml (contact time 20 min) strongly inhibited, while 3 ng/ml (contact time 5 min) significantly poten-

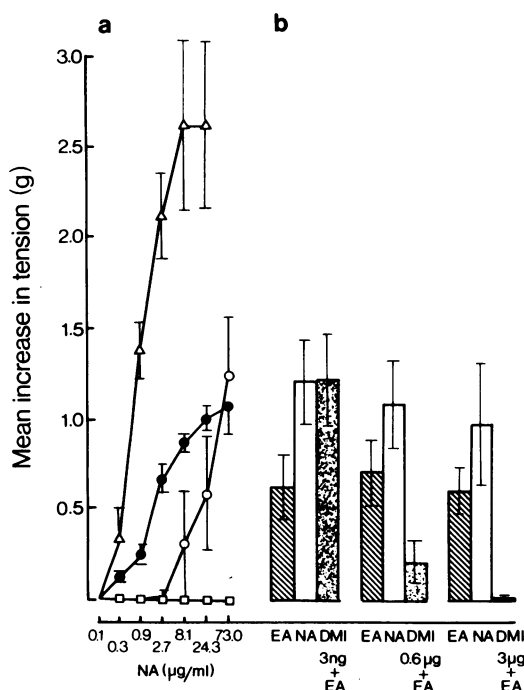


Fig. 4 (a) The effect of three doses of desipramine (DMI) on the response of guinea-pig vas deferens to noradrenaline (NA). (●) Control (8 experiments); (Δ) 3 ng/ml, (□) 0.6 µg/ml, and (○) 3 µg/ml of DMI (each point is the average of 4 experiments). (b) The effect of three doses of DMI (concentration shown per ml) on the response of guinea-pig vas deferens to ethacrynic acid (EA, 200 µg/ml). Response to NA (24 µg/ml) was obtained before addition of DMI. Control response to EA was obtained in contralateral vas deferens. Each column is the mean of 4–7 experiments. Vertical lines indicate s.e. mean.

tiated the responses to EA. Parallel changes were observed in the responses of vasa deferentia from another 8 animals to NA after pretreatment with desipramine. These are shown in Figure 4a.

Pretreatment of the vasa with hexamethonium (100 µg/ml) did not inhibit the response of the tissue to EA (400 µg/ml, 4 paired experiments), while it completely prevented that to nicotine (10 µg/ml). Hexamethonium did not prevent the potentiating effect of cocaine.

Phentolamine is capable of inhibiting the response of the tissue to muscarinic agents (Benfrey & Grillo, 1963; Boyd, Burnstock, Campbell, Jowett, Oshea & Wood, 1963) and reserpine might nonspecifically inhibit the response of the tissue to agonists (Fleming & Trendelenburg, 1961; Westfall & Fleming, 1968).

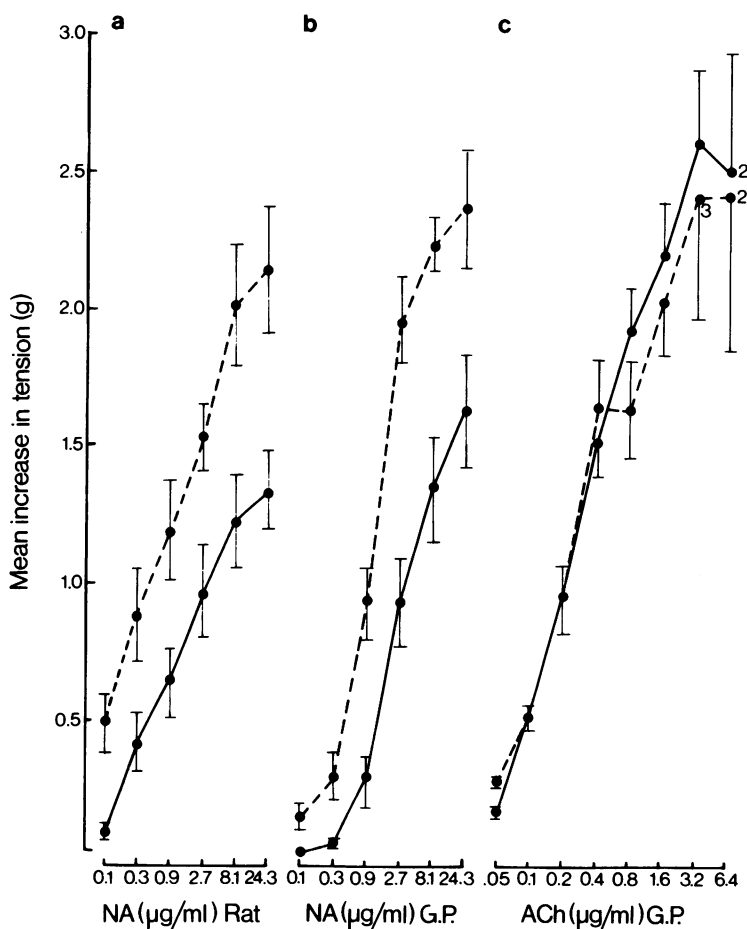


Fig. 5 Isometric contractions of rat (a) and guinea-pig (G.P.) (b & c) vas deferens induced by noradrenaline (NA) (a & b) or acetylcholine (ACh) (c). Continuous line, before; broken line, after treatment with ethacrynic acid (200 $\mu\text{g/ml}$). Each point is the mean of 4 (a & c) or 8 (b) experiments unless otherwise indicated. Vertical lines indicate s.e. mean.

This necessitated the study of the effect of atropine on the EA-induced contractions. Pretreatment of the tissue with atropine (0.1 $\mu\text{g/ml}$), which completely prevented the response to acetylcholine (3 $\mu\text{g/ml}$), had no effect on the response of the vasa to EA. The results were compared with EA-induced contractions of the contralateral vasa deferentia.

In 8 experiments, dose-response curves to NA were obtained before and after EA tachyphylaxis developed (Figure 5b). The tissue showing tachyphylaxis was incubated with EA (200 $\mu\text{g/ml}$) for a further 3 min before NA was added. As shown in Fig. 5b, EA enhanced the responses of the vasa deferentia to all doses of NA ($P < 0.05$). In similar

experiments (4 animals) tachyphylaxis to EA did not change the dose-response curve to acetylcholine significantly (Figure 5c).

EA does not cause any contraction in rat vas deferens, which might reasonably be interpreted as being due to the absence of NA release, or a release which is too slow in this species. The rat is also resistant to some other effects of EA (Duggan & Noll, 1966; Dow & Irvine, 1967). Therefore, experiments were carried out to see whether EA enhances the response to NA in this species. The results are shown in Figure 5a. In the presence of 200 $\mu\text{g/ml}$ of EA the contractile responses to NA were significantly potentiated ($P < 0.02$, 4 experiments each dose).

Discussion

The ability of reserpine and phentolamine to inhibit the contraction due to EA suggests that the contraction is indirectly mediated through the release of catecholamines. Wakade & Krusz (1972) have shown that reserpine, at the dose used in the present experiments, depletes the vasa completely of catecholamine. Development of tachyphylaxis is also in accord with the suggestion of an indirect action of EA.

The inability of atropine and hexamethonium to inhibit the response of the vasa to EA excludes the possibility of action on muscarinic and nicotinic receptors of the nervous elements.

The mechanism of release does not seem to involve the same neuronal uptake mechanism as is responsible for the indirect sympathomimetic action of tyramine, since there is no cross tachyphylaxis to tyramine, and cocaine and low concentrations of desipramine, far from abolishing the effect actually potentiate it. This argument, however, is not as strong in the vas deferens as it would be in other tissues in which cocaine inhibits the effect of tyramine (Furchgott, Kirpekar, Rieker & Schwab, 1963). In the rat vas deferens some reports suggest that cocaine does block tyramine (Greenberg & Long, 1971) whereas other workers (Barnett, Symchowicz & Taber, 1968; Barnett, Staub & Symchowicz, 1969) found that cocaine neither blocks the mechanical response to tyramine nor its ability to release radioactive NA. Barnett *et al.* (1968) attributed their results to the presence of specific tyramine receptors on the terminal adrenergic nerves which were not sensitive enough to cocaine. The present observation that cocaine and desipramine do not block the effect of EA and that there is no cross tachyphylaxis to tyramine suggests that, in releasing NA, EA is acting on yet another receptor site separate both from that acted on by cocaine and by tyramine.

It is of course possible that EA releases NA by a quite different mechanism from other indirect

sympathomimetics perhaps by a mechanism related to its known abilities to influence ion movements (Batra & Daniel, 1970). Either depolarization of the nerve endings or perhaps a more specific increase in Ca^{++} entry might result in transmitter release. In support of this is the observation that EA can apparently release acetylcholine from cholinergic nerves in the rabbit jejunum since the contraction in that tissue is blocked by atropine or morphine (Khoiyi & Salami, unpublished observations).

The inhibitory effect of higher doses of desipramine (Fig. 4b) on the contractile response to EA is due to its α -adrenoceptor blocking property. In these concentrations it also inhibits significantly the effect of NA on guinea-pig vas deferens (Figure 4a). Similar reports on the α -receptor blocking property of desipramine and related compounds on other organs or species have been published (Ursillo & Jacobson, 1965; Barnett *et al.*, 1968; Westfall, 1973).

The ability of EA to potentiate the response to NA without altering the response to acetylcholine is consistent with an interference with the NA neuronal uptake mechanism. A similar potentiation by EA of the action of NA on aortic strips has previously been reported (Baggio & Poggioli, 1969) and Torsti *et al.* (1968) have shown on the rat heart a decreased uptake of radioactive NA. Nevertheless, the observation in the present experiments that cocaine and desipramine greatly potentiate the response to EA suggests that any interference with NA uptake is incomplete.

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