SPECIFICITY OF ABOLITION OF DIBENAMINE BLOCK OF α-ADRENERGIC RECEPTORS BY TRYPSIN

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Trypsin restores the noradrenalin contraction of the isolated vas deferens of rats when preliminarily blocked by dibenamine. This effect is not due to sensitization of the smooth muscles to noradrenalin.

The β-haloethylamines produce prolonged blocking of α-adrenergic receptors by alkylation of the anion point [7]. In experiments on the isolated vas deferens of guinea pigs and on rats with recording of the arterial pressure, trypsin has been shown to abolish the blocking of the effect of catecholamines produced by dibenamine, SY-28, and L_2, but not the effects of acetylcholine and histamine. Other enzymes investigated (phosphates, phosphodiesterase, and papain) had no action similar to that of trypsin [5]. Moran et al. [6] consider that the abolition by trypsin of the blocking of α-adrenergic receptors in a strip of rabbit's aorta produced by SY-28 is a nonspecific effect, because trypsin increases the contractions of control specimens, i.e., it sensitizes smooth muscles to the action of noradrenalin. They also found that phosphodiesterase and subtilisin possess a similar action to that of trypsin.

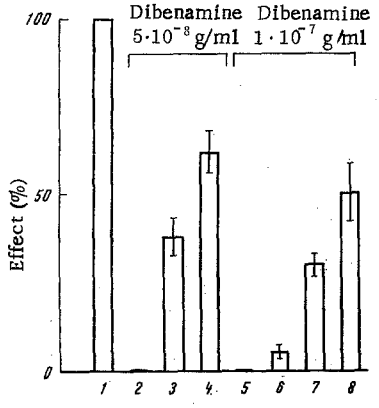

Fig. 1. Restoration of noradrenalin contractions of the rat vas deferens by trypsin after dibenamine block: 1) control standard contraction of vas by noradrenalin (1×10^{-6} g/ml); 2, 5) reaction to noradrenalin after treatment with dibenamine; 3, 6) restoration of contractions of vas to noradrenalin after treatment with trypsin, 8×10^{-2} $TU^{Hb}_{35.5°}$; 4, 7) the same, after treatment with trypsin, 2.4×10^{-1} $TU^{Hb}_{35.5°}$. Values of $M \pm m$ shown.

The investigation described below was carried out to study the specificity of the deblocking action of trypsin on the dibenamine block of α-adrenergic receptors of smooth muscles.

EXPERIMENTAL METHOD

Experiments were carried out on the isolated vas deferens of rats, kept in Krebs' solution with aeration at 37°C. Contractions of the vas were recorded under isotonic conditions by an Engelmann's lever (1 : 10).

The standard magnitude of contraction of the smooth-muscle preparation to noradrenalin in a concentration of 1×10^{-6} g/ml was determined. Next, dibenamine was added to the bath in a concentration of 5×10^{-8} or 1×10^{-7} g/ml for 10 min. The degree of blocking was determined from the decrease in amplitude of the standard noradrenalin contraction (1×10^{-6} g/ml). In the absence of a contraction, trypsin was added to the bath (8×10^{-2}, 2.4×10^{-1}, and 4×10^{-1} $TU^{Hb}_{35.5°}$) for 10 min, and after rinsing, noradrenalin was added in a concentration of 1×10^{-6} g/ml. Restoration of the contractions by trypsin was expressed

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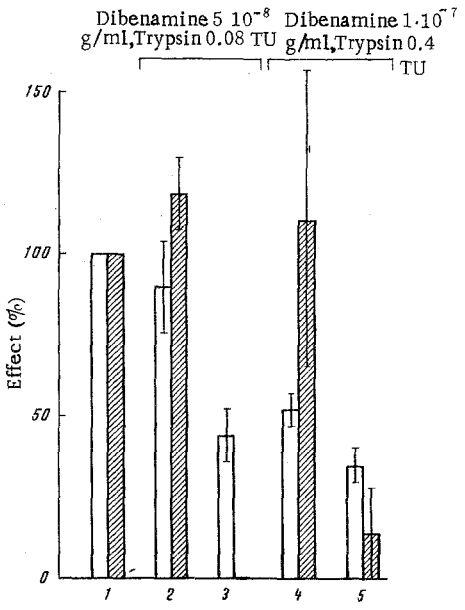

Fig. 2. Effect of preliminary treatment with trypsin on reactions of vas deferens to noradrenalin and serotonin, and on deblocking action of trypsin: 1) control contractions of vas to noradrenalin and serotonin in concentrations of 1×10^{-6} and 1×10^{-5} g/ml respectively; 2,4) reactions to noradrenalin and serotonin after preliminary treatment with trypsin; 3,5) deblocking action of trypsin. Values of $M \pm m$ given. Unshaded columns show effects of noradrenalin; shaded columns show effects of serotonin.

as a percentage of the amplitude of the original standard contraction. Similar experiments were carried out with serotonin (1×10^{-5} g/ml).

To test the specificity of the deblocking action of trypsin, the smooth-muscle preparation of the vas deferens, after standardization of the noradrenalin effect, was treated with trypsin (8×10^{-2} or 4×10^{-1} $TU^{Hb}_{35.5°}$) for 10 min. The amplitude of contraction of the vas deferens in noradrenalin was then determined, after which the vas was treated with dibenamine (5×10^{-8} or 1×10^{-7} g/ml) and the deblocking action of trypsin was investigated in the two above-mentioned concentrations. Similar series of experiments were carried out also with ribonuclease (10 and 50 mg per bath with a volume of 15 ml).

EXPERIMENTAL RESULTS AND DISCUSSION

In the concentrations used, dibenamine totally and irreversibly suppressed contractions of the rat vas deferens caused by noradrenalin in a concentration of 1×10^{-6} g/ml. Trypsin abolished the dibenamine block of the α-adrenergic receptors. The higher the concentration of trypsin, the greater the degree of restoration of the noradrenalin effects. To abolish the stronger block due to dibenamine in a concentration of 1×10^{-7} g/ml, correspondingly higher concentrations of trypsin were required (Fig. 1). The dibenamine block of serotonin effects under these experimental conditions is not abolished by trypsin.

In a special series of experiments, the action of trypsin in a concentration of 8×10^{-2} $TU^{Hb}_{35.5°}$ on the smooth-muscle preparation did not change the sensitivity of smooth muscles of the rat vas deferens to noradrenalin, but in high concentrations (4×10^{-1} $TU^{Hb}_{35.5°}$) trypsin actually reduced the noradrenalin contractions of the duct. The effects of serotonin were slightly potentiated after preliminary treatment with trypsin (Fig. 2).

Ribonuclease, a phosphodiesterase [1], in the concentrations used, did not abolish the dibenamine block of the α-adrenergic receptors of smooth muscles.

These results indicate that trypsin specifically abolishes the block of adrenergic receptors caused by dibenamine.

Trypsin hydrolyzes peptide, amide, and ester bonds formed by the carboxyl groups of L-arginine or L-lysine [2,3]. Abolition of the dibenamine block of adrenergic receptors by trypsin may be due to hydrolysis of the ester bond formed by alkylation of the terminal carboxyl group of these amino acids by dibenamine during blocking of the adrenergic receptors. From this point of view, the anion point of the α-adrenergic receptors, as has been suggested previously [4, 8], is the carboxyl group belonging probably to arginine or to lysine.

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