

THE BLOCKING ACTION OF PRONETHALOL ON THE CONTRACTION OF ISOLATED SPLEEN STRIPS PRODUCED BY ADRENALINE, ACETYLCHOLINE OR ISOPROTERENOL

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It has been reported that spleen strips contract with an addition of adrenaline (1-3), noradrenaline (3), acetylcholine (1-3), histamine (1-2) or 5-hydroxytryptamine (2) to the bath medium. The adrenaline-induced contraction of the spleen strips can be blocked by alpha-adrenergic blocking agents (1-4). According to Bickerton (4), isoproterenol, which stimulates beta-adrenergic receptors, also contracts the spleen strips and the isoproterenol-induced contraction is specifically blocked by a beta-adrenergic blocking agent, dichloroisoproterenol (5).

The present paper describes that pronethalol, one of the beta-adrenergic blocking agents originally reported by Black and Stephenson (6), inhibits the contraction of spleen strips produced by adrenaline, acetylcholine or isoproterenol, in different species of animals.

MATERIALS AND METHODS

Spleen strips obtained from cats, kids and rabbits were used for the present experiments. Cats were anesthetized with ether, and kids and rabbits were knocked with a blow on the head. After these animals were killed by cutting carotid arteries, spleens were removed through an abdominal incision and put in beakers containing Krebs-Henseleit solution. The blood was cleared off the isolated spleens as much as possible. Spleen strips 25 to 30 mm long and 2 to 3 mm wide were prepared from these spleens, and were suspended in organ baths containing 10 ml of Krebs-Henseleit solution, maintained at 38°C and bubbled with 100% oxygen. Every strip included a strip of spleen capsule. Isotonic contractions of the spleen strip were recorded with a kymograph.

Responses of the spleen strips to agonists (adrenaline, acetylcholine and isoproterenol) were measured before and after exposure to various concentrations of an antagonist (pronethalol). Each agonist remained in the bath until the full contraction was attained. Washout of the drugs was accomplished by draining and refilling the bath with fresh Krebs-Henseleit solution at least three times. Each agonist was added at 15 minute intervals to the medium. Pronethalol was added after washout of the agonist. The agonist was added again 5 minutes after the addition of pronethalol without washing.

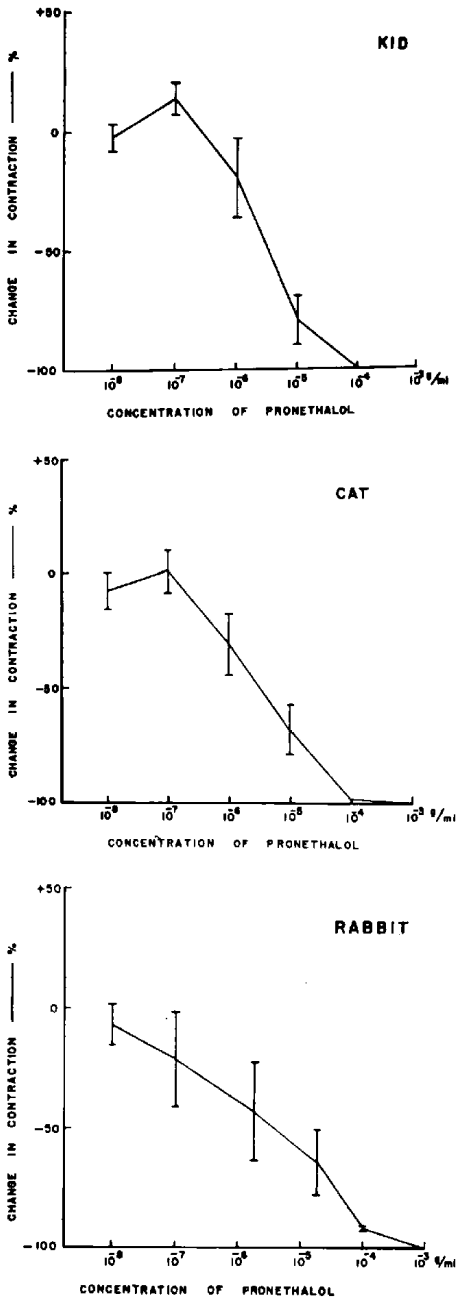


FIG. 1. The effects of various concentrations of pronethalol on the submaximal contraction of spleen strips produced by adrenaline in kids, cats and rabbits. Changes in the responses to adrenaline after exposure to pronethalol were shown by expressing the rate of increase or decrease in percentages of the control responses. I : Standard error

Effect of pronethalol was shown by expressing the rate of increase in percentages of the control responses to agonists.

Drugs used were adrenaline hydrochloride, acetylcholine hydrochloride, isoproterenol sulfate and 2-isopropylamino-1-[2-naphthyl] ethanol hydrochloride (pronethalol). All concentrations in the text refer to final concentrations (g/ml) of salts in the bath medium.

RESULTS

1. The adrenaline-induced contraction (Fig. 1)

In 7 kid spleen strips, adrenaline in the concentrations ranging from 10^{-8} to 5×10^{-8} produced a submaximal contraction. The adrenaline-induced contraction of the spleen strips was not affected with a previous addition of 10^{-8} of pronethalol. In the presence of 10^{-7} of pronethalol, the response to adrenaline was augmented by about 15% compared with the control response. However, increases in the concentrations of pronethalol above 10^{-6} resulted in gradual decrease in the response of the spleen strips to adrenaline. A complete blockade of the adrenaline-induced contraction occurred with an addition of 10^{-4} of pronethalol.

In 6 cat spleen strips, adrenaline in the concentrations ranging from 10^{-8} to 10^{-7} produced a submaximal contraction. A previous addition of 10^{-7} of pronethalol did not affect the degree of contraction. Increases in the concentrations of pronethalol above 10^{-6} resulted in decreases in the adrenaline-induced contraction proportional to the concentrations of pronethalol. A complete blockade of the adrenaline-induced contraction was observed with an addition of 10^{-3} of pronethalol.

In the experiment with 3 rabbit spleen

strips, adrenaline in the concentrations ranging from 2×10^{-8} to 3×10^{-8} produced a submaximal contraction. A previous addition of pronethalol in the concentrations above 10^{-8} did not augment the adrenaline-induced contraction but inhibited the contraction proportional to the concentrations of pronethalol. The adrenaline-induced contraction was completely blocked with an addition of 10^{-3} of pronethalol.

2. The acetylcholine-induced contraction (Fig. 2)

Acetylcholine in a concentration of 10^{-7} produced a submaximal contraction in 6 kid spleen strips. A previous addition of 10^{-8} of pronethalol, slightly augmented the acetylcholine-induced contraction. In the presence of 10^{-7} of pronethalol, the response to acetylcholine returned to the control level. However, increases in the concentrations of pronethalol above 10^{-6} resulted in gradual decreases in the response to acetylcholine. With an addition of 10^{-4} of pronethalol, the acetylcholine-induced contraction was completely blocked.

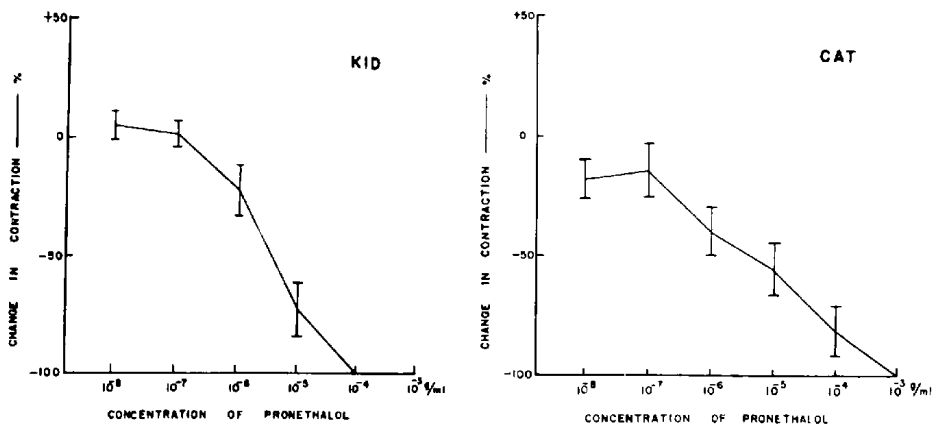


FIG. 2. The effects of various concentrations of pronethalol on the submaximal contraction of spleen strips produced by acetylcholine in kids and cats. Changes in the responses to acetylcholine after exposure to pronethalol were shown by expressing the rate of increase or decrease in percentages of the control responses.

I: Standard error

In 6 cat spleen strips, a submaximal contraction was observed with an addition of acetylcholine in the concentrations ranging from 10^{-5} to 5×10^{-5} . Increases in the concentrations of pronethalol above 10^{-8} resulted in gradual decreases in the response to acetylcholine of the spleen strips. An augmentation of the response did not occur. In the presence of 10^{-3} of pronethalol, the spleen strips no longer developed detectable contraction with an addition of acetylcholine.

3. The isoproterenol-induced contraction (Fig. 3)

Isoproterenol in the concentrations ranging from 10^{-5} to 5×10^{-5} produced a submaximal contraction in 6 kid spleen strips. A significant augmentation of the isoproterenol-induced contraction was observed with a previous addition of 10^{-8} , 10^{-7} or 10^{-6} of pronethalol. However, 10^{-5} of pronethalol markedly reduced the response of the spleen

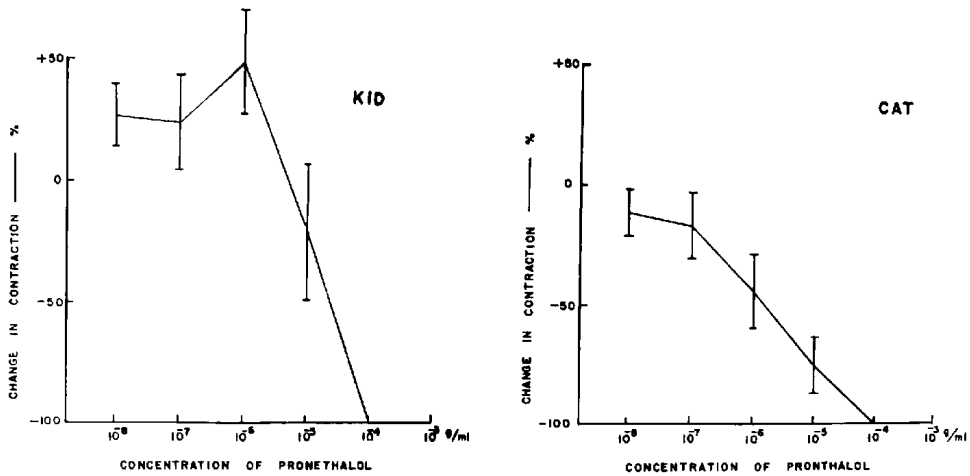


FIG. 3. The effects of various concentrations of pronethalol on the submaximal contraction of spleen strips produced by isoproterenol in kids and cats. Changes in the responses to isoproterenol after exposure to pronethalol were shown by expressing the rate of increase or decrease in percentages of the control responses.

I : Standard error

strips to isoproterenol, and 10^{-4} of pronethalol completely blocked the response to isoproterenol.

In 6 cat spleen strips, isoproterenol in the concentrations ranging from 10^{-5} to 2×10^{-5} produced a submaximal contraction. Pronethalol in the concentrations above 10^{-8} reduced but did not augment the isoproterenol-induced contraction. The response to isoproterenol was completely blocked with a previous addition of 10^{-4} of pronethalol.

DISCUSSION

In the presence of high concentrations of pronethalol (about 10^{-5} or more), contractions of spleen strips produced by adrenaline, acetylcholine or isoproterenol were markedly inhibited in kids, cats and rabbits. Adrenaline has been considered to contract the spleen strips by stimulating the alpha-adrenergic receptors in the spleen, because the adrenaline-induced contraction of the spleen is specifically blocked by alpha-adrenergic blocking agents (1-4). The mechanism of contraction of the spleen produced by acetylcholine is still obscure, but an hypothesis (7, 8) proposing that acetylcholine releases adrenaline and noradrenaline in the spleen, is favorable to the explanation of the contraction of spleen produced by acetylcholine. Although it was demonstrated that isoproterenol contracted the spleen strips, about 1,000 times higher concentration of isoproterenol was required to produce the contraction compared with that of adrenaline. This suggests that isoproterenol-induced contraction of the spleen strips is not due to its stimulating action on the beta-adrenergic receptors. It may be that isoproterenol in high concentrations stimulates the alpha-adrenergic receptors to contract the spleen strips.

The concentration of pronethalol which inhibited the responses to adrenaline, acetylcholine or isoproterenol by about 50% compared with the control responses, was usually

between 10^{-5} to 10^{-4} . This evidence suggests that the inhibitory action of pronethalol on the contraction of spleen strips produced by adrenaline, acetylcholine or isoproterenol, is not due to the specific blocking action of pronethalol on the beta-adrenergic receptors. Two explanations would be possible on the mechanism of blocking action of pronethalol upon the contraction produced by adrenaline, acetylcholine or isoproterenol. One is that pronethalol may inhibit the alpha-adrenergic receptors which might be stimulated by adrenaline, acetylcholine or isoproterenol. The other is that pronethalol may inhibit the contraction *per se* but not receptors. It is also probable that the both factors are concerned with the production of blocking action of pronethalol on the contraction of spleen strips induced by adrenaline, acetylcholine or isoproterenol.

The responses of the spleen strips to each of the agonists after exposure to low concentrations of pronethalol (below 10^{-6}) were not constant. In kid spleen strips, an augmentation of the responses to the three agonists was observed in every experiment with an addition of pronethalol. But in cats, an inhibition of the responses without significant augmentation was observed with a previous addition of pronethalol. In rabbit spleen strips, pronethalol did not augment but inhibited the response to adrenaline. Therefore, it is likely that differences in the animal species is an important factor with regard to the augmentation of the responses of spleen strips to adrenaline, acetylcholine or isoproterenol, in the presence of low concentrations of pronethalol.

SUMMARY

The effects of an antagonist (pronethalol) on the contraction of spleen strips produced by agonists (adrenaline, acetylcholine and isoproterenol) were studied in the preparations prepared from kids and rabbits. The same study was done using adrenaline as an agonist also in rabbits.

1. High concentrations of pronethalol (about 10^{-5} g/ml or more) inhibited the responses of the spleen strips to each of the agonists in all the experiment done.

2. The augmentation of the responses of spleen strips to each of the agonists occurred in the presence of low concentrations of pronethalol (below 10^{-6} g/ml) in kids. However, a significant augmentation of the responses to the three agonists was not observed with a previous addition of low concentrations of pronethalol, in the experiment with cats. In rabbits, the augmentation of the response to adrenaline with an addition of low concentrations of pronethalol, was not observed either.

3. It is suggested that the inhibitory effects of high concentrations of pronethalol on the contraction of spleen strips produced by adrenaline, acetylcholine or isoproterenol, are not due to the specific action of pronethalol on the beta-adrenergic receptors.

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