A STUDY ON THE CONTRACTION OF SPLEEN STRIPS IN KIDS AND DOGS, WITH SPECIAL REFERENCE TO THE CHOLINERGIC AND ADRENERGIC RECEPTORS

SHIZUKO TAKANO

Department of Pharmacology, Fukushima Medical College, Fukushima

Received for publication May 22, 1969

It has been reported that the splenic smooth muscles of several species of animals can be contracted with noradrenaline (1-4), adrenaline (1, 3-9), isoproterenol (1, 3-5), acetylcholine (5-8), serotonin (7), histamine (1, 7), tyramine (7), angiotensin (10), or BaCl₂. The existence of alpha-adrenergic receptors has been confirmed in the splenic smooth muscles in the cat, dog, kid, rabbit, and human (1, 5, 8, 11). The stimulation of the alpha-adrenergic receptors in the spleen produces contraction of the organ. It is of interest that isoproterenol, which stimulates the beta-adrenergic receptors, contracts the spleen in cats and mice, but the drug relaxes it in mice when the concentration was very low. The isoproterenol-induced contraction of the spleen in cats can be inhibited by dichloroisoproterenol or dibenamine (1), and the isoproterenol-induced relaxation of it in mice can be inhibited by MJ-1999 or propranolol (4). Two explanations are possible for these findings; one is that there are beta-adrenergic receptors in the spleen and the other is that isoproterenol stimulates the alpha-adrenergic receptors in the spleen to produce contraction. As to the mechanism of the contraction of the spleen produced by acetylcholine, Burn and Rand proposed hypothesis in 1960: acetylcholine releases noradrenaline and adrenaline in the spleen, and these catecholamines produce the contraction (12, 13).

The present experiments were undertaken to examine whether there were beta-adrenergic receptors in the spleen, and to confirm the hypothesis proposed by Burn and Rand.

MATERIALS AND METHODS

Spleen strips obtained from kids and dogs were used for the present study. The spleen strips 25 to 35 mm long and 2 to 3 mm wide were prepared from dogs anesthetized with the intravenous injection of 30 mg/kg of pentobarbital sodium, and also from kids of about three months of age killed by a blow on the heads. Each spleen strip was suspended in the organ bath containing 10 ml Krebs solution maintained at 38°C and bubbled with 100% oxygen. Isotonic contractions of the spleen strips at 0.4 to 1.3 g tension were recorded with a kymograph at 10.9 times amplification. Each strip included the spleen capsule.

The contraction-producing drugs (agonists) employed were noradrenaline hydro-
chloride, acetylcholine hydrochloride, isoproterenol hydrochloride, and barium chloride. The antagonists employed were dibenamine hydrochloride, atropine sulfate, and propranolol hydrochloride.

The agonists were kept in contact with the spleen strip until full contraction was attained. After an agonist was washed out by rinsing the strip with fresh Krebs solution at least three times, the strip was exposed to an antagonist usually for a period of five minutes, then the agonist was added again to the bath without washing out the antagonist. In the experiment in which dibenamine was used as an antagonist, the spleen strip was exposed to the drug for fifteen minutes before adding agonists because of the slow onset of action of dibenamine. In order to obtain a constant response of the strip, the agonists were added at 10 to 15-minute-intervals to the bath medium. All the concentrations in the text refer to the final concentrations (g/ml) of salts in the bath medium.

RESULTS

1. The contraction of spleen strips produced by agonists (Table 1)

Noradrenaline, acetylcholine, isoproterenol, and BaCl₂ were used as agonists to produce a contraction of the spleen strips. Table 1 shows concentrations of the agonists needed to produce a moderate degree of contraction of the spleen strips (6 to 33.7 mm-deflection on a smoked paper). Both the spleen strips taken from kids and dogs responded similarly to each of the agonists. Noradrenaline and acetylcholine usually contracted the strips of both species of animals in concentrations below $5 \times 10^{-7}$. Although the upper limit of the concentration of acetylcholine to produce contraction of the spleen strip is higher than that of noradrenaline (Table 1), that is because there were two exceptional experiments in which the strip did not respond to acetylcholine until the concentration of $2 \times 10^{-6}$ or $5 \times 10^{-6}$ was reached. Isoproterenol and barium chloride contracted the spleen strips only in higher concentrations compared with those of noradrenaline and acetylcholine. In both species of animals, isoproterenol and barium chloride did not contract the strips in concentrations below $2 \times 10^{-6}$.

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Kid spleen strip</th>
<th>Dog spleen strip</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine hydrochloride</td>
<td>$2 \times 10^{-6} \sim 2 \times 10^{-3}$</td>
<td>$10^{-4} \sim 5 \times 10^{-3}$</td>
</tr>
<tr>
<td>Noradrenaline hydrochloride</td>
<td>$4 \times 10^{-6} \sim 5 \times 10^{-6}$</td>
<td>$8 \times 10^{-4} \sim 2 \times 10^{-7}$</td>
</tr>
<tr>
<td>Isoproterenol hydrochloride</td>
<td>$2 \times 10^{-6} \sim 5 \times 10^{-6}$</td>
<td>$2 \times 10^{-4} \sim 5 \times 10^{-6}$</td>
</tr>
<tr>
<td>BaCl₂·2H₂O</td>
<td>$10^{-3} \sim 2 \times 10^{-3}$</td>
<td>$3 \times 10^{-4} \sim 10^{-3}$</td>
</tr>
</tbody>
</table>

II. Actions of antagonists on agonist-induced contraction (Table 2-A, 2-B)

1. Atropine

In kids and dogs the acetylcholine-induced contraction of the spleen strips was completely blocked by the preliminary addition of $10^{-7}$ of atropine. The noradrenaline- or barium-induced contractions, however, were not blocked by $10^{-7}$ of atropine in both
species of animals. Accordingly, atropine seems to possess a specific blocking action on acetylcholine-induced contraction of the spleen strip.

2. **Dibenamine**

In the presence of $10^{-7}$ of dibenamine, the contraction of the spleen strips produced by noradrenaline or isoproterenol was markedly reduced in kids and dogs. In the kids, the noradrenaline- and isoproterenol-induced contractions were reduced to $8 \pm 3.7\%$ and to $12 \pm 8\%$ of the control responses respectively, with the preliminary addition of dibenamine. In the dogs, the contraction of spleen strips produced by either noradrenaline or isoproterenol was completely blocked by $10^{-7}$ of dibenamine. But the acetylcholine- or barium-induced contraction was not inhibited significantly with the addition of dibenamine in both species of animals. From these findings, it is evident that $10^{-7}$ of dibenamine blocks specifically the splenic contraction which is to be induced by noradrenaline or isoproterenol. If the hypothesis proposed by Burn and Rand is correct, the acetylcholine-induced contraction of the spleen should be inhibited by dibenamine.

3. **Propranolol**

With the addition of $10^{-7}$ of propranolol, isoproterenol produced a contraction of the spleen strip to the degree of $80 \pm 8\%$ of the control response in kids. In the dog spleen
strips, however, the response to isoproterenol was slightly augmented in the presence of $10^{-7}$ of propranolol. With the addition of the high concentration of $10^{-4}$ of propranolol, the contraction of the spleen strips produced by noradrenaline, isoproterenol, or barium was greatly reduced in both species of animals. In seven kid-spleen strips out of sixteen, a transient contraction of the strip was produced by $10^{-4}$ of propranolol, but the isoproterenol-induced contraction was not blocked completely by propranolol in kids. In the dog-spleen strips, $10^{-4}$ of propranolol per se did not cause any contraction, but it abolished almost completely the contraction which was to be produced by barium or isoproterenol. The noradrenaline-induced contraction was also inhibited with that concentration of propranolol by about 12% compared with that of the control.

DISCUSSION

The spleen strips obtained from kids and dogs responded to each of the agonists used in the present experiments to a similar degree. The spleen strip obtained from the dog, however, was more stable in its base line and more reproducible in response to the drugs than that obtained from the kid. The mouse spleen is considered to be more sensitive to noradrenaline and to adrenaline than that of the kid or dog. The mouse spleen can be contracted with these drugs in the low concentration of $10^{-7}$ M (4). The concentration of isoproterenol sufficient to relax the mouse spleen has been reported to be $5 \times 10^{-10}$ M (4), but it is not sufficient to change the isotonic tonus of the spleen strips in kids and dogs.

The presence of the alpha-adrenergic receptors in the splenic smooth muscles has been confirmed by several investigators (1, 5, 6, 11, 14). In kid- and dog-spleen strips, isoproterenol seems to stimulate the alpha-adrenergic receptors to produce contraction: in the first place, the concentrations of isoproterenol about 1,000 times higher than those of noradrenaline are required to produce a moderate degree of contraction; in the second place, the isoproterenol- and noradrenaline-induced contraction can be inhibited specifically by dibenamine in the concentration of $10^{-6}$; and in the third place, propranolol in the concentration of $10^{-7}$ does not inhibit the isoproterenol-induced contraction in the dog or slightly inhibits in the kid. The blocking action of propranolol in high concentrations seems to be nonspecific, for the high concentration of $10^{-4}$ of propranolol blocked the contraction of the spleen strips which is to be produced by noradrenaline, isoproterenol, and barium. Bickerton demonstrated that noradrenaline, adrenaline, and isoproterenol stimulated the alpha-adrenergic receptors in cats using the receptor protection method (1, 9). In the mouse spleen, the presence of the beta-adrenergic receptors was reported by Ignarro et al. (4). They observed that the concentration as low as $5 \times 10^{-10}$ M of isoproterenol caused relaxation of the mouse spleen, but higher concentrations than $5 \times 10^{-9}$ M caused contraction. The relaxation was inhibited by MJ-1999 or propranolol, and the contraction was inhibited by phenoxybenzamine. This evidence may indicate that the alpha-adrenergic receptors contribute to the contraction and the beta-adrenergic receptors contribute to the relaxation of the spleen in the mouse. From the results of the present experiment, it is not likely that acetylcholine liberates noradrenaline from a store around
the nerve endings to contract the spleen. It is likely that acetylcholine has its own receptors which differ from those of noradrenaline in the spleen. The present study did not demonstrate the existence of beta-adrenergic receptors in the spleen of kids and dogs, the beta-adrenergic receptors, which are found in the mouse spleen, may not exist in the kid and dog spleens.

SUMMARY

The spleen strips taken from kids and dogs were used for the present study. The effects of antagonists (atropine, dibenamine, and propranolol) on the contraction of the spleen strips produced by agonists (noradrenaline, acetylcholine, isoproterenol and barium) were investigated.

1. The acetylcholine-induced contraction was blocked by $10^{-7}$ of atropine, but not by $10^{-7}$ of dibenamine.

2. The noradrenaline-induced contraction was blocked by $10^{-7}$ of dibenamine, but not by $10^{-7}$ of atropine. The contraction was also blocked by propranolol in the high concentration of $10^{-4}$.

3. The isoproterenol-induced contraction was effectively blocked by $10^{-7}$ of dibenamine. Propranolol in the concentration of $10^{-7}$ did not block the contraction in the dog, but it did by about 20% in kids. The contraction, however, was effectively blocked by the high concentration of $10^{-4}$ of propranolol, which also inhibited markedly the noradrenaline- or barium-induced contraction.

4. The barium-induced contraction was not blocked by $10^{-7}$ of atropine, or that of dibenamine, but was completely blocked by $10^{-4}$ of propranolol.

From the above results, it is suggested that acetylcholine contracts the spleen strips not by releasing noradrenaline but by affecting its own receptors, and that isoproterenol contracts the strips not by stimulating the beta-adrenergic receptors but by stimulating the alpha-adrenergic receptors. The existence of the beta-adrenergic receptors could not be confirmed in kids and dogs.

REFERENCES

8) Saad, K.: *Quart. J. Pharm.* 8, 31 (1935)

