THE EFFECT OF STIMULATION OF \( \alpha \)- AND \( \beta \)-ADRENERGIC RECEPTORS IN THE ISOLATED RAT JEJUNUM

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It has been reported that stimulation of either \( \alpha \)- or \( \beta \)-adrenergic receptors produces relaxation of the canine ileum, mouse ileum, rat duodenum, human ileum, and human colon (1-6). On the other hand, it has been found that adrenaline contracts the terminal portion of the guinea-pig ileum (7-9). Chrusciel and Pojda (10) reported a triphasic intestinal response to adrenaline in the rat, which consisted of an initial relaxation and a secondary increase in the tone followed by a third relaxation.

The present work was undertaken to study the effect of stimulation of \( \alpha \)- and \( \beta \)-adrenergic receptors on the rat jejunum.

MATERIALS AND METHODS

Rats of either sex weighing from 130 to 260 g were used. The animals were killed by bleeding after a blow on the head and the jejunum was rapidly removed. Segments of the jejunum about 2 cm in length were suspended in an organ bath containing 20 ml of Gaddum solution. The organ bath was bubbled with 100% oxygen, and the temperature was maintained at 27°C throughout the experiments. Isotonic contractions of the jejunum segments at 0.7 g resting tension were recorded with a kymograph at 8.5 times amplification. The drugs used in this study are as follows.

Agonists: Adrenaline hydrochloride, isoproterenol hydrochloride and phenylephrine hydrochloride.

Antagonists: Dibenamine hydrochloride, propranolol hydrochloride, dl-4-(2-hydroxy-3-isopropylaminopropoxy)-indole (LB-46), \( d(-) \) and \( l(+) \) isomers of N-isopropyl-p-nitrophenylethanamine hydrochloride (\( d(-) \) INPEA and \( l(+) \) INPEA).

The concentrations (g/ml) of these drugs in the text are expressed in terms of the final concentrations of salts in the organ bath.

RESULTS

Adrenaline usually produced a triphasic response after treatment of the jejunum with a \( \beta \)-adrenergic blocking agent.

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Table 1. Effects of antagonists on \( h_1 \) and \( h_2 \) (see Fig. 1) produced by agonists.

| Antagonists     | Agonists          | Adrenaline (5 \( \times \) 10\(^{-4} \) g/ml) | Isoproterenol (5 \( \times \) 10\(^{-2} \) g/ml) | Phenylephrine (5 \( \times \) 10\(^{-2} \) g/ml) |
|-----------------|-------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
|                 | \( h_1 \) (mm)    | \( h_2 \) (mm)                                | \( h_1 \) (mm)                                | \( h_2 \) (mm)                                |
| Propranolol     | Control           | \( -20.96 \) ± 2.57                           | \( -18.01 \) ± 3.58                         | \( -4.09 \) ± 1.45                           |
|                 | (8 \( P^* < 0.01 \)) |                                 | (10 \( P^* < 0.001 \))                         | (8 \( P^* < 0.01 \))                         |
|                 | After propranolol | \( -9.02 \) ± 1.09                            | \( -2.92 \) ± 2.03                          | \( -4.84 \) ± 1.22                           |
|                 |                   | \( +10.53 \) ± 2.94                          | \( +2.03 \) ± 1.03                           | \( +16.38 \) ± 3.01                           |
|                 |                   | \( +2.94 \)                                   |                                               |                                               |
| LB-46           | Control           | \( -13.72 \) ± 2.91                            | \( -20.48 \) ± 4.04                         | \( -15.02 \) ± 1.85                           |
|                 | (5 \( \times \) 10\(^{-2} \) g/ml) |                                 |                                               |                                               |
|                 | (8 \( P^* < 0.01 \)) |                                 |                                               |                                               |
|                 | After LB-46       | \( -4.80 \) ± 1.37                            | \( 0 \) ± 1.90                               | \( 7.30 \) ± 1.43                            |
|                 |                   | \( +12.33 \) ± 1.90                          |                                               |                                               |
|                 |                   | \( +1.37 \)                                   |                                               |                                               |
| \( -(-) \) INPEA| Control           | \( -20.43 \) ± 1.85                            | \( -21.00 \) ± 2.75                         | \( -9.33 \) ± 1.66                           |
|                 | (10 \( \times \) 10\(^{-4} \) g/ml) |                                 |                                               |                                               |
|                 | (8 \( P^* < 0.001 \)) |                                 |                                               |                                               |
|                 | After \( -(-) \) INPEA | \( -8.66 \) ± 3.15                           | \( 0 \) ± 3.15                               | \( -8.79 \) ± 1.50                           |
|                 |                   | \( +14.83 \) ± 3.15                          |                                               |                                               |
|                 |                   | \( +1.49 \)                                   |                                               |                                               |
| \( (+) \) INPEA | Control           | \( -19.61 \) ± 3.94                            | \( -21.69 \) ± 3.11                         | \( -12.91 \) ± 2.24                           |
|                 | (10 \( \times \) 10\(^{-4} \) g/ml) |                                 |                                               |                                               |
|                 | (8 \( P^* < 0.2 \)) |                                 |                                               |                                               |
|                 | After \( (+) \) INPEA | \( -17.68 \) ± 5.54                           | \( -19.34 \) ± 3.2                           | \( -9.97 \) ± 3.2                             |
|                 |                   | \( +6.54 \) ± 2.76                           |                                               |                                               |
|                 |                   | \( +3.61 \)                                   |                                               |                                               |
| Dibenamine      | Control           | \( -25.59 \) ± 5.58                            | \( -14.15 \) ± 2.68                         | \( -8.26 \) ± 3.67                           |
|                 | (2.5 \( \times \) 10\(^{-7} \) g/ml) |                                 |                                               |                                               |
|                 | (8 \( P^* < 0.05 \)) |                                 |                                               |                                               |
|                 | After dibenamine  | \( -35.03 \) ± 2.79                           | \( 0 \) ± 3.04                               | \( -1.49 \) ± 0.86                           |
|                 |                   |                                               |                                               |                                               |

The degrees of \( h_1 \) and \( h_2 \) are expressed by mm change (average ± standard error) on smoked paper. The number of preparations tested is shown in parentheses.

\( P^* \): Probability determined by Student's \( t \) test utilizing paired data analysis.
agent like propranolol, LB-46, or D(-) INPEA. Fig. 1 shows a schematic illustration of the adrenaline-induced response in the presence of β-adrenergic blocking agents, in which the maximum relaxation is expressed by \( h_1 \) and maximum contraction is expressed by \( h_9 \). As the adrenaline-induced relaxation without adding β-adrenergic blocking agents has also a similar pattern of response to that shown in Fig. 1, \( h_1 \) and \( h_9 \) of the control response to adrenaline were measured and compared with those obtained after the addition of each antagonist. Responses to isoproterenol and to phenylephrine were also measured in terms of \( h_1 \) and \( h_9 \), and were compared with those obtained after the addition of antagonists. The effects of antagonists on \( h_1 \) and \( h_9 \) are summarized in Table 1.

1. Effects of antagonists on the response to adrenaline (Table 1, Fig. 2)

Adrenaline in a concentration of \( 5 \times 10^{-4} \) caused a relaxation of the jejunum. During the relaxation, it was occasionally observed that the tone of the jejunum increased slightly, then decreased again. When \( 2.5 \times 10^{-7} \) of propranolol, \( 5 \times 10^{-7} \) of LB-46, or \( 10^{-6} \) of D(-) INPEA was added to the bath solution about 5 minutes before the addition of adrenaline, the maximum relaxation \( (h_1) \) was inhibited and the maximum contraction \( (h_9) \) was increased. In the experiments where adrenaline-induced contraction was not

![Fig. 2. Change in response to \( 5 \times 10^{-8} \) g/ml of adrenaline (Adr) in the presence of \( 2.5 \times 10^{-7} \) g/ml of propranolol (Rat A), \( 5 \times 10^{-7} \) g/ml of LB-46 (Rat B), \( 10^{-6} \) g/ml of D(-)INPEA (Rat C), \( 10^{-6} \) g/ml of L(+)-INPEA (Rat D) and \( 2.5 \times 10^{-7} \) g/ml of dibenamine (Rat E).]
observed in the absence of β-adrenergic blocking agents, the phase of contraction was produced by the previous addition of each of the three β-adrenergic blocking agents.

The previous addition of $10^{-8}$ of $L(-)$ INPEA decreased $h_1$ slightly and increased $h_2$ slightly, but these changes were not statistically significant. When $2.5 \times 10^{-7}$ of dibenamine was added to the bath about 30 minutes before the addition of adrenaline, the degree of $h_1$ increased significantly.

2. Effects of antagonists on the response to isoproterenol (Table 1, Fig. 3)

Isoproterenol in a concentration of $5 \times 10^{-7}$ caused a relaxation of the jejunum. When $2.5 \times 10^{-7}$ of propranolol was added to the bath 5 minutes before the addition of isoproterenol, the relaxation-producing effect of isoproterenol was almost completely blocked. Similar results were obtained with the previous addition of $5 \times 10^{-7}$ of LB-46 or $10^{-8}$ of $D(-)$ INPEA. In the presence of propranolol or $D(-)$ INPEA, isoproterenol occasionally produced a very slight contraction.

Unlike $D(-)$ INPEA, $L(+)$ INPEA in a concentration of $10^{-6}$ little affected the isoproterenol-induced relaxation of the jejunum. When $2.5 \times 10^{-7}$ of dibenamine was added to the bath solution 30 minutes before the addition of isoproterenol, the degree of isoproterenol-induced relaxation was significantly increased.

![Fig. 3. Change in response to $5 \times 10^{-7}$ g/ml of isoproterenol (Iso) in the presence of $2.5 \times 10^{-7}$ g/ml of propranolol (Rat F), $5 \times 10^{-7}$ g/ml of LB-46 (Rat G), $10^{-6}$ g/ml of $D(-)$ INPEA (Rat H), $10^{-6}$ g/ml of $L(+)\text{INPEA}$ (Rat I) and $2.5 \times 10^{-7}$ g/ml of dibenamine (Rat J).]
3. Effects of antagonists on the response to phenylephrine (Table 1, Fig. 4)

Phenylephrine in a concentration of $5 \times 10^{-7}$ caused a relaxation followed by a contraction in most of the experiments. (In 6 out of a total of 46 experiments, phenylephrine did not produce the biphasic response but only a contraction. In 2 out of the 46 experiments, phenylephrine produced only a relaxation.) The response to phenylephrine resembled that to adrenaline in the presence of propranolol, LB-46, or D(-)INPEA. Propranolol $2.5 \times 10^{-7}$ did not affect $h_1$ but reduced $h_2$ significantly. When $5 \times 10^{-7}$ of LB-46 was added to the bath before the addition of phenylephrine, $h_1$ was inhibited. The previous addition of D(-)INPEA $10^{-6}$ little affected $h_1$ and increased $h_2$.

On the other hand, D(+)-INPEA inhibited $h_1$ significantly and did not affect $h_2$. When $2.5 \times 10^{-7}$ of dibenamine was added to the bath 30 minutes before the addition of phenylephrine, both $h_1$ and $h_2$ were almost completely inhibited.

Fig. 4. Change in response to $5 \times 10^{-7}$ g/ml of phenylephrine (Phe) in the presence of $2.5 \times 10^{-7}$ g/ml of propranolol (Rat K), $5 \times 10^{-7}$ g/ml of LB-46 (Rat L), $10^{-6}$ g/ml of D(-)INPEA (Rat M), $10^{-6}$ g/ml of L(+)-INPEA (Rat N) and $2.5 \times 10^{-7}$ g/ml of dibenamine (Rat O).
DISCUSSION

There is a controversy about the mechanism of the relaxation of intestinal smooth muscle produced by catecholamines. According to Ahlquist (11), stimulation of the α-adrenergic receptors in the intestine produces relaxation of the organ. Inhibition of the gastrointestinal tract, however, was shown to be mediated by α- as well as β-adrenergic receptors (1). Nickerson et al. (12) reported that dibenamine did not affect the relaxation produced by adrenaline in the rat and rabbit small intestines in vitro and in the non-pregnant cat uterus in situ.

The present experiments demonstrated that the relaxation of the rat jejunum produced by adrenaline was not decreased but increased by the previous addition of dibenamine. When β-adrenergic blocking agents such as propranolol, LB-46, and D(-)INPEA were previously added to the bath, adrenaline produced an initial phase of relaxation followed by a secondary phase of contraction. In most cases, the secondary phase of contraction was not apparent in the absence of β-adrenergic blocking agents, but occasionally adrenaline alone caused a relaxation followed by a slight contraction. In these cases, the contraction induced by adrenaline was augmented by the previous addition of propranolol, LB-46 or D(-)INPEA. Since L(+)-INPEA, which is considered to be devoid of a β-adrenergic blocking action (13), did not affect the response of the jejunum to adrenaline, it seemed that the effects of the β-adrenergic blocking agents used in this study on the adrenaline-induced relaxation was specific. Similar results were obtained when isoproterenol was used as an agonist. Isoproterenol produced a relaxation of the jejunum, which was almost completely blocked by propranolol, LB-46, or D(-)INPEA. The degree

\[ \text{Rat 130 g } \]

\[ \begin{align*}
\text{Adrenaline} & \quad \text{Propranolol} \\
\text{Adrenaline} & \quad \text{Propranolol}
\end{align*} \]

\[ \text{Dibenamine} \]

\[ \text{Propranolol} \]

\[ \text{Adrenaline} \]

Fig. 5. Incomplete blockade of the adrenaline-induced relaxation by dibenamine, $2.5 \times 10^{-7} \text{ g/m}l$ and propranolol, $2.5 \times 10^{-7} \text{ g/m}l$. The concentration of adrenaline added is $5 \times 10^{-7} \text{ g/m}l$. 


of relaxation induced by isoproterenol was increased by the previous addition of dibenamine. This may be because isoproterenol has a weak stimulating-action on the \( \alpha \)-adrenergic receptors. In view of these results, it is assumed that stimulation of the \( \beta \)-adrenergic receptors produces an initial relaxation followed by a secondary contraction, and that stimulation of the \( \alpha \)-adrenergic receptors produces a relaxation without a contraction in the rat jejunum. If this assumption is correct, it is inconceivable that adrenaline initiates relaxation or contraction of the jejunum in the presence of both \( \alpha \)- and \( \beta \)-adrenergic blocking agents. Adrenaline, however, relaxed the jejunum pretreated with dibenamine and propranolol (Fig. 5). The reason for this is not clear, but the antagonistic interaction between \( \alpha \)- and \( \beta \)-adrenergic blocking agents (1) might be one of the important factors in modifying the response to adrenaline. Phenylephrine, which is known to stimulate mainly the \( \alpha \)-adrenergic receptors, caused a relaxation followed by a contraction in most cases. This response caused by phenylephrine was almost completely blocked by the previous addition of dibenamine. The result with phenylephrine well accords with the view that stimulation of the \( \alpha \)-adrenergic receptors produces an initial relaxation followed by a secondary contraction.

The action of phenylephrine on the intestine, however, has been reported in different ways by different investigators. Although phenylephrine was reported to produce only a relaxation in the dog and human ileum and the rat duodenum (1, 4, 5, 14, 15), it was shown to cause a contraction of the guinea-pig ileum and occasionally of the rat stomach (16, 17). The present study demonstrated that phenylephrine produced both relaxation and contraction, and that the effects of \( \beta \)-adrenergic blocking agents (propranolol, LB-46, \( \sigma \)-INPEA) on the phenylephrine-induced response were not consistent. In Table 1, it can be seen that LB-46 inhibits \( h_{2} \) response to phenylephrine, but the inhibition seems to be a result of the inhibition of \( h_{1} \) (Fig. 4). Kohli (16) and Innes et al. (17) reported that the contraction induced by phenylephrine might be due to the stimulation of the serotonin-receptors. In order to interpret these findings, more studies would be needed about the action of phenylephrine on the serotonin-receptors as well as on the adrenaline-receptors.

**SUMMARY**

The present study was undertaken to determine the nature of the adrenergic receptor mechanism in the rat jejunum in vitro. Adrenaline, isoproterenol, and phenylephrine were used as agonists and dibenamine, propranolol, LB-46, \( \sigma \)-INPEA, and \( \tau \)-INPEA were used as antagonists.

1. Isoproterenol \( 5 \times 10^{-3} \) (g/ml) and adrenaline \( 5 \times 10^{-8} \) caused a relaxation of the isolated rat jejunum. During the relaxation induced by adrenaline, it was occasionally observed that the tone of the jejunum increased slightly, then decreased again.

2. Phenylephrine \( 5 \times 10^{-7} \) caused a relaxation followed by a contraction in most of the experiments.

3. The relaxation induced by adrenaline or isoproterenol was not decreased but
increased by the previous addition of dibenamine $2.5 \times 10^{-7}$. But the response to phenylephrine was almost completely blocked by the previous addition of dibenamine.

4. Adrenaline usually produced a relaxation followed by a contraction after the treatment of the jejunum with propranolol $2.5 \times 10^{-7}$, LB-46 $5 \times 10^{-7}$ or $\text{D}(-)\text{INPEA} 10^{-6}$. But $\text{L}(+)\text{INPEA} 10^{-6}$ little affected the response to adrenaline or isoproterenol.

5. The effects of $\beta$-adrenergic blocking agents (propranolol, LB-46, $\text{D}(-)\text{INPEA}$) on the response to phenylephrine were not consistent. $\text{L}(+)\text{INPEA}$ slightly inhibited the phase of relaxation of the phenylephrine-induced response.

The results of the present study suggest that the stimulation of the $\alpha$-adrenergic receptors produces a relaxation followed by a contraction, and that the stimulation of the $\beta$-adrenergic receptors produces only a relaxation in the rat jejunum.

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REFERENCES
1) AHLQUIST, R.P. AND LEVY, B.: J. Pharmac. exp. Ther. 127, 146 (1959)
2) LEVY, B.: J. Pharmac. exp. Ther. 127, 150 (1959)
3) TAKAGI, K., OSADA, E., TAKAYANAGI, I. AND TAGA, F.: Archs int. Pharmacodyn. Thér. 168, 212 (1967)
4) LEVY, B.: Archs int. Pharmacody. Thér. 170, 418 (1967)
5) BENNETT, A.: Nature, Lond. 208, 1289 (1965)
6) BUCKNELL, A. AND WHITNEY, B.: Br. J. Pharmac. Chemother. 23, 164 (1964)
7) KAWAI, A.: J. Osaka Med. Coll. 22, 263 (1964)
8) KAWAI, A.: J. Osaka Med. Coll. 22, 271 (1964)
9) MUNRO, A.F.: J. Physiol. 118, 171 (1952)
10) CHRUŚCIEL, T.L. AND POJDA, S.M.: Experientia 24, 1227 (1968)
11) AHLQUIST, R.P.: Am. J. Physiol. 153, 586 (1948)
12) NICKERSON, M. AND GOODMAN, L.S.: J. Pharmac. exp. Ther. 89, 167 (1947)
13) MURMANN, W. AND GAMBA, A.: Boll. Chim. Farm. 106, 101 (1967)
14) LEVY, B.: Br. J. Pharmac. Chemother. 27, 277 (1966)
15) COUPER, I.M. AND TURNER, P.: Br. J. Pharmac. Chemother. 36, 213p (1969)
16) KOHLI, J.D.: Br. J. Pharmac. Chemother. 32, 273 (1968)
17) INNES, I.R. AND KOHLI, J.D.: Br. J. Pharmac. Chemother. 35, 383 (1969)