The concept of two adrenergic receptor mechanisms or α- and β-adrenergic receptor systems, originally postulated by Ahlguist (1) is presently accepted. Furchgott (2) has presented evidence suggesting that there may be different types of receptors in the β-class. This conclusion was based on differences in relative potencies of isoprenaline, epinephrine, norepinephrine and phenylephrine in variety of isolated organs.

On the other hand, some partial agonists in a series of cholinergic drugs contract the isolated taenia caecum (or taenia coli) of the guinea pig and at the same time the contraction which they produce is inhibited by their non-specific inhibitory action, so that maximal responses to partial agonists are smaller than that to full agonists (3−5). Furthermore, ephedrine which was classified as an adrenergic partial agonist relaxes the taenia caecum and at the same time the relaxation which it produces is antagonized by its own excitatory action, so maximal relaxation by it is smaller than that by a full agonist, epinephrine (5). DCI was also reported as an adrenergic partial agonist (6−8). However, mode of action of DCI is not precisely examined.

The purpose of this paper is to compare the activities of some β-adrenergic blockers in various organs and to determine if there are different types of the β-adrenergic receptors in the organs. Furthermore, mode of action of DCI which is known as a dualist on some organs was precisely tested.

METHODS

A piece of the taenia caecum (or taenia coli), atrium and tracheal muscle (9) from the male guinea pig (300 to 350 g in body weight) and a piece of the jejunum and the fundus of the stomach from the male rat (150 to 200 g in body weight) prepared by Vane's method (10) were suspended in a 30 ml organ bath. The responses of these preparations to the drugs were recorded through an isotonic lever.

The responses of the uterus from the castrated rat (150 to 200 g in body weight) suspended in the same organ bath was isometrically recorded through a mechano-electrical transducer (RCA-5734).

Locke Ringer solution gassed with a mixture of 95% O₂ and 5% CO₂, and kept at 32°C was used as a bath fluid. Locke Ringer solution used contained 9.0 g of NaCl, 0.4 g of KCl, 0.2 g of CaCl₂, 0.2 g of MgCl₂, 0.2 g of NaHCO₃ and 0.5 g of glucose in a litre.
In some experiments, a modified Locke Ringer solution was used, in which NaCl and NaHCO₃ were replaced by KCl and KHCO₃ (5). This modified Locke Ringer solution was called "K-Locke Ringer solution" in this paper.

The agonistic activity of a drug was expressed as the pD₂ value or the negative logarithm of the concentration which produced 50% of the maximal response of the smooth muscle. The competitive antagonistic activity of a drug was expressed as the pA₂ value, which was calculated from the parallel shift of a concentration action curve of the agonist (11, 12). However, relaxing activity of DCI tested on the smooth muscles was expressed as the pD'₂ value or the negative logarithm of the concentration necessary to produce 50% of the maximum relaxation (12).

The concentration action curves of the agonist or isoprenaline on the taenia caecum, tracheal muscle, uterus and fundus were cumulatively obtained. The concentration action curve of isoprenaline on the isolated atrium was obtained as follows. The responses to two suitable concentrations of isoprenaline were obtained. The concentration action curve of isoprenaline was drawn by plotting the responses increased by isoprenaline which were measured as shown in Fig. 1 A-E.

Experiments with a β-adrenergic blocker: After a constant control response to an agonist had been obtained, the agonist was given to the preparation which had been treated with an antagonist for 3 minutes beforehand. In the experiments with the atrium, contraction

FIG. 1. Responses of the isolated atrium to isoprenaline and to DCI in the presence and absence of propranolol.

Time : 1 second.
A, B, C, D : Response to isoprenaline. F, G : response to DCI. C, D, G : response in the presence of propranolol (3×10⁻⁴ M). E : concentration action curves of isoprenaline in the presence and absence of propranolol (3×10⁻⁷ M). Note that the responses to isoprenaline were blocked by propranolol (3×10⁻⁷ M) but those to DCI were slightly reduced.
increased by two concentrations of isoprenaline in the presence of the β-blocker was also
drawn by the above mentioned way. The pA₂ value of the blocker was calculated from
the parallel shift of the concentration action curve of isoprenaline (Fig. 1 A-E).

Experiments with dibenamine: Cumulative concentration action curves were obtained
using a similar technique to that described by van Rossum and Ariens (13). When the
jejunum of the rat gave constant response to acetylcholine, concentration action curve of
acetylcholine was made. After this, the jejunum was incubated with dibenamine \(3 \times 10^{-5}
M\) for 20 minutes. After washing out thoroughly, concentration action curve of acetyl-
choline was again obtained.

Each result in this paper is the mean of at least 7 experiments.

Drug used: DCI (dichloroisopropynorepinephrine hydrochloride), ICI-50172 (4-\((2-
hydroxy-3-isopropylaminopropoxy)acetanilide hydrochloride\), propranolol hydrochloride,
pronethalol hydrochloride, TMI (2-isopropylamino-1-(2-(1'2'3'4'-tetrahydroxy)naphthyl
ethanol hydrochloride) (Fig. 2), isoprenaline hydrochloride, acetylcholine chloride, atro-
pine sulfate, papaverine hydrochloride and dibenamine hydrochloride. TMI was report-
ed as a β-adrenergic blocker by us (14).

RESULTS

Subdivision of the β-adrenergic receptors

As shown in Table 1, ICI-50172 selectively inhibited the responses of the taenia caecum
and the atrium but not the responses of the tracheal muscle, uterus and fundus to isopre-
naline. These results are similar to those reported by Levy and Wilkenfeld (15). The
other blockers, such as propranolol, pronethalol, DCI and TMI antagonized the responses
### TABLE 1. The pA₂ values of the β-adrenergic blockers against isoprenaline.

|       | Guinea pig | Rat       |
|-------|------------|-----------|
|       | Taenia caecum | Trachea | Atrium | Uterus | Fundus |
| Propranolol | 7.2(0.2) | 7.2(0.1) | 7.5(0.3) | 7.6(0.4) | 6.3(0.2) |
| Pronethalol | 6.1(0.2) | 6.3(0.2) | 6.4(0.3) | 6.4(0.4) | 5.3(0.2) |
| ICI-50172   | 5.8(0.1) | 4.5(0.3) | 5.8(0.4) | 4.2(0.3) | 4.1(0.3) |
| DCI        | 5.8(0.3)  | 5.6(0.4) | 6.1(0.4) | —     | —     |
| TMI        | 5.7(0.3)  | 5.6(0.3) | —       | 5.8(0.4) | —     |
| Isoprenaline | 6.8(0.1)** | 7.4(0.1)** | 6–7** | 9.5(0.1)** | 7.0(0.1)** |

*; index for papaverine like activity or pD₂
**; index for agonistic activity or pD₂
(); standard deviation

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**Fig. 3.** Responses of the taenia caecum and of the tracheal muscle to isoprenaline and to DCI in the presence and absence of propranolol.

Upper trace: responses of the taenia caecum. Lower trace: responses of the tracheal muscle.

Note that the responses to isoprenaline were antagonized by propranolol (10⁻⁶ M) but those to DCI were not.
of the preparations with the exception of the fundus of the stomach. After all, only the response of the fundus to isoprenaline was resistant to the action of all the β-blockers used in this paper. These results are summarized in Table 1.

**Analysis of the action of DCI**

The tracheal muscle and taenia caecum: DCI relaxed the tracheal muscle and taenia caecum. The relaxation produced by DCI was little affected by $10^{-5}$M of propranolol, which was enough concentration to antagonize the action of isoprenaline (Fig. 3). After it was confirmed that isoprenaline, DCI and papaverine relaxed the taenia caecum suspended in the normal Locke Ringer solution, the same taenia caecum was depolarized with the K-Locke Ringer solution. The taenia caecum depolarized by K$^+$ was still relaxed by DCI and papaverine but not by isoprenaline. The behavior of DCI was quite similar to that of papaverine. These phenomena are demonstrated in Fig. 4.

The atrium: DCI increased the contraction of the atrium. The action of DCI was not blocked by $3 \times 10^{-7}$M of propranolol, which was enough concentration to block the response to isoprenaline, as shown in Fig. 1F and G.

**Relation between effective concentrations of an agonist and of an antagonist and concentration of drug receptor in the smooth muscle**

1) **Theory**

The reaction of an agonist A with its receptor R may be written as

$$A + R \rightleftharpoons AR$$

(1)
The mass action law for steady state conditions gives

$$[A] = \frac{K_A}{[Rt]} \frac{[AR]}{[R]} - 1$$  \hspace{1cm} (2)$$

Where \([A]\) represents the molar concentration of the drug \(A\) in equilibrium with receptor \(R\). The other brackets \([R]\), \([AR]\) and \([Rt]\) indicate the concentrations of the free receptors and of the drug-receptor complexes and the total concentrations of the receptors. \(K_A\) is the dissociation constant.

If the same quantity of \([AR]\) is formed, the same response to the drug \(A\) must be observed. In the case that \([Rt]\) becomes smaller after the isolated organ is, for instance, incubated with dibenamine, does the effective concentration of an agonist change? So, if \([Rt]\) = \(n_1\) and \([A_1]\) is the molar concentration necessary to form a certain quantity of \([AR]\),

$$[A_1] = \frac{K_A}{n_1 - 1}$$  \hspace{1cm} (3)$$

Furthermore, if \([Rt]\) = \(n_2\), \([A_2]\) or the molar concentration necessary to form the same quantity of \([AR]\) as that mentioned above is written as

$$[A_2] = \frac{K_A}{n_2 - 1}$$  \hspace{1cm} (4)$$

Therefore, ratio of two effective concentrations \(\frac{[A_1]}{[A_2]}\) is given as

$$\frac{[A_1]}{[A_2]} = \frac{n_2 - 1}{n_1 - 1}$$  \hspace{1cm} (5)$$

Equation (5) indicates that concentration of the drug necessary to make the same response is large, if the total concentration of the receptors \([Rt]\) is small.

If the agonist \(A\) and its inhibitor \(B\) compete for the same receptor \(R\), the reaction may be written as

\(R + A \rightleftharpoons AR\) and \(R + B \rightleftharpoons BR\)  \hspace{1cm} (6)$$

The mass action law for steady state condition gives

$$\frac{[B]}{K_B} + 1 = \frac{[A]}{K_A} \left(\frac{[Rt]}{[AR]} - 1\right)$$  \hspace{1cm} (7)$$

Where \([B]\) and \([BR]\) represent the concentrations of the drug and of the drug-receptor complexes. \(K_B\) shows the dissociation constant.

If the molar concentration of the competitive inhibitor \(B\) necessary to produce a certain parallel shift of the concentration action curve of the agonist \(A\) is \([B_1]\) and \([Rt]\) = \(n_1\), (in this case the molar concentration of the agonist \(A\) is \([A_1]\) (see Fig. 5)),

$$\frac{[B_1]}{K_B} + 1 = \frac{[A_1]}{K_A} (n_1 - 1)$$  \hspace{1cm} (8)$$

Furthermore, if \([B_2]\) is the molar concentration of the competitive inhibitor \(B\) necessary to the same parallel shift of the curve of the agonist \(A\) as that mentioned above and \([Rt]\) = \(n_2\), (in this case the molar concentration of the agonist \(A\) is \([A_2]\) (See Fig. 5)),

$$\frac{[B_2]}{K_B} + 1 = \frac{[A_2]}{K_A} (n_2 - 1)$$  \hspace{1cm} (9)$$

$$\frac{[B_1]}{K_B} + 1 = \frac{[B_2]}{K_B} (n_2 - 1)$$  \hspace{1cm} (10)$$
Fig. 5. Effect of a competitive inhibitor and "total" concentration of its receptors: theoretical concentration action curves. Since dibenamine irreversibly combines with the acetylcholine receptors, the concentration of the receptors in the organ treated with dibenamine is smaller than that in the untreated organ.

(A1): the molar concentration of the agonist A to produce the same response y of the untreated organ.

(A1'): the molar concentration of the agonist A to produce the same response y of the untreated organ in the presence of the competitive inhibitor B.

(A2): the molar concentration of the agonist A to produce the same response y of the dibenamine-treated organ.

(A2'): the molar concentration of the agonist A to produce the same response y of the dibenamine-treated organ in the presence of the competitive inhibitor B.

(B1): the molar concentration of the competitive inhibitor B to produce the same parallel shift X of the concentration action curve of the agonist A on the untreated organ.

(B2): the molar concentration of the competitive inhibitor B to produce the same parallel shift X of the concentration action curve of the agonist tested on the dibenamine-treated organ. (B1) = (B2) finally.

\[
[B_1] + 1 = \frac{[A_1]}{[A_2]} (n_2 - 1) \tag{9}
\]

The following equation is then obtained;

\[
\frac{[B_1]}{[B_2]} = \frac{[A_1]}{[A_2]} (n_2 - 1) \tag{10}
\]

Substituting equation (5) into equation (10) and rearranging gives

\[
\frac{[B_1]}{[B_2]} + 1 = \frac{[A_1]}{[A_2]} \frac{[A_1]}{[A_2]} \tag{11}
\]

If \[\frac{[A_1]}{[A_2]} = \frac{[A_1]}{[A_2]} \] (see Fig. 5),

\[
\frac{[B_1]}{[B_2]} + 1 = 1
\]

Then
Equation (12) indicates that the molar concentration of the competitive inhibitor B necessary to produce the same shift of the concentration action curve of the agonist A is same, irrespective of the total concentration of receptors in an organ.

2) Experiment

It is well known that dibenamine irreversibly combines with the acetylcholine receptor, so that the concentration of the acetylcholine receptor in the preparation treated with dibenamine is smaller than that in the untreated preparation.

The concentration action curve of acetylcholine was parallely shifted towards about 100 times higher concentrations by the 20 minute incubation of the rat jejunum with dibenamine $(3 \times 10^{-5} \text{ M})$, as shown in Fig. 6. The pA$_2$ value of atropine against acetylcholine was $8.84 \pm 0.2$ (mean $\pm$ standard deviation) on the untreated jejunum and $8.76 \pm 0.2$ on the jejunum treated with dibenamine $(3 \times 10^{-5} \text{ M})$ for 20 minutes. These pA$_2$ values were calculated from the parallel shift shown in Fig. 6. Two pA$_2$ values obtained were practically same.

DISCUSSION

It is indicated in this paper that if a nature of drug receptors is identical, the effective concentration of an agonist depends upon a concentration of the receptors in the organ but the effective concentration of a competitive inhibitor does not. Therefore, the fact that
the pA₄ values of β-adrenergic blockers estimated on the various organs are significantly
different from each others indicates that there are different types of the β-adrenergic recep-
tors within these organs. Table 1 shows that there may be at least three types of the
β-receptors even within the limited number of organs investigated in this paper.

1) The β-receptor in the taenia caecum and atrium belongs to the first group, which
is selectively blocked by ICI-50172.

2) The second group consists of the β-receptor in the tracheal muscle and uterus
to which ICI-50172 is less sensitive. The β-receptors in both two groups are blocked by
propranolol, pronethalol, DCI and TMI.

3) Only the β-receptor in the fundus of the rat stomach belongs to the third group.
The β-receptor in this group is less sensitive to the β-adrenergic blockers than those in other
groups are.

It is not determined if these conclusions indicate that the different types of the β-
receptors vary in their chemical constitution, since pharmacologists cannot yet isolate and
chemically characterize the receptors. There is still the possibility that all the β-receptors
may be identical molecules, but that their properties are influence by interaction with
surrounding molecules in the macromolecular structures, such as cell membrane, in which
the receptors are located. If these macromolecular structures, such as cell membranes
vary somewhat from one organ to another, this may alter the properties of the β-receptors
for interaction with agonists and antagonists.

It was already reported that DCI had also agonistic action on some organs and was
classified as a partial agonist (6-8). However, the inhibitory action of DCI on the smooth
muscle was not affected by propranolol and contraction of the atrium increased by DCI
was slightly reduced by propranolol. Furthermore, the smooth muscle depolarized by
K⁺ was relaxed by DCI and papaverine but not by isoprenaline. It is given as a con-
clusion that DCI is not a real partial agonist, since the mimetic action is not mediated
through the β-adrenergic receptor and relaxation of the smooth muscle by DCI is due to
its papaverine like action.

**SUMMARY**

The pA₄ values of five β-adrenergic blockers against isoprenaline were obtained on
the taenia caecum, tracheal muscle and atrium from the guinea pig and the uterus and
the fundus of the stomach from the rat. It was concluded from the results that there are
at least three types of the β-adrenergic receptors even within the above organs. The β-
adrenergic receptor in the taenia caecum and atrium of the guinea pig belongs to the first
group, the β-receptor in the tracheal muscle of the guinea pig and the rat uterus to the
second group and the β-receptor in the fundus of the rat stomach to the third group. DCI
had both β-adrenolytic and agonistic actions. Since agonistic action of DCI was not
mediated through the β-adrenergic receptor, DCI is thought not to be a real partial agonist.
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