THE ACTION OF SYMPATHOMIMETIC AMINES AND A NEW *BETA*-ADRENERGIC BLOCKING AGENT (4- (2-HYDROXY-3-ISOPROPYLAMINOPROPOXY)- INDOLE) ON THE ISOLATED TRACHEA OF GUINEA PIGS

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The report that dichlorisoprenaline, DCI, the first introduced *beta*-adrenergic receptor blocking agent, could block *beta*-adrenergic receptors initiated a research for other *beta*-receptor blocking agents.

Thereafter, several compounds, such as pronethalol [Black and Stephenson (1)], propranolol [Black *et al.* (2)], H56/28 [Åblad *et al.* (3)] and others, have been described as capable of producing more potensive and selective blockade of *beta*-adrenergic receptor sites.

Recently a new *beta*-adrenergic receptor antagonist, 4-(2-hydroxy-3-isopropylamino-propoxy)-indole (LB-46) (4), has been introduced. This compound is a basic indole with a side-chain characteristic of *beta*-adrenergic receptor blocking agents.

Investigation was performed to make clear an antagonistic effect of this compound against isoprenaline, adrenaline and noradrenaline in comparison with the above-mentioned *beta*-adrenergic receptor blocking agents.

METHODS

Guinea pigs weighing from 300 to 350 g were used. The trachea was removed from the guinea pigs sacrificed by a blow to the head, and washed several times with the Tyrode's solution. One end of the trachea was tightly attached to one end of the capillary tube of the modified Jamieson apparatus (5) and the other end to the U-tube of this apparatus.

The preparation was suspended in a 20 ml organ bath of 37°C and gassed with a mixture of 95% oxygen and 5% carbon dioxide.

An addition of constricting or relaxing agents to the organ bath caused an increase or decrease in column height, dependent on the change in the volume of the tracheal segment used.

A response of the tracheal muscle is proportional to the square root of the change in height of the manometer fluid. Therefore, the square root of an estimated value was plotted against the log dose to give a concentration-response curve.

All the experiments were designed to yield the log concentration-response curves for each of catecholamines before and after an application of the *beta*-adrenergic blocking agents. Each of the *beta*-adrenergic blocking agents was given just 10 minutes before an addition of catecholamines.

The change in height of the manometer fluid was read just 5 minutes after an addition of catecholamines. The maximum relaxation was determined by an application of catecholamines in a suitable dose at the beginning of the experiment.

The following compounds were employed.

4-(2-Hydroxy-3-isopropylaminopropoxy)-indole, LB-46, 1-(*o*-allylphenoxy)-3-isopropylamino-2-propanol, H56/28, and propranolol.

RESULTS

Log concentration-response curves for isoprenaline, adrenaline and noradrenaline were figured respectively without and with the application of the *beta*-adrenergic blocking agents. The potency of the *beta*-adrenergic blocking agents was compared with each other by the analysis of the curves obtained.

1. Antagonistic action of LB-46 against isoprenaline

Fig. 1 illustrates the antagonistic potency of LB-46 in concentrations of 10^{-7} , 10^{-6} and 10^{-5} against isoprenaline.

The results of Fig. 1 can be expressed as those in Fig. 2 by replotting them in another way (6).

FIG. 1. Log concentration-response curves of the antagonistic action of LB-46 in concentrations of 10^{-7} , 10^{-6} and 10^{-5} against isoprenaline. The dotted lines represent the curves to be expected if isoprenaline in such concentrations was applied.

Ordinate : % Relaxation (y)

Abscissa : Log concentration of isoprenaline ($\text{Log } x$)

FIG. 2. Log concentration-response curves of the antagonistic action of LB-46 in concentrations of 10^{-7} , 10^{-6} and 10^{-5} against isoprenaline.

Ordinate : Relaxation $\left(\text{Log } \frac{y}{y_{\text{max}} - y} \right)$

Abscissa : Log concentration of isoprenaline ($\text{Log } x$)

FIG. 3. Log concentration-response curves of the antagonistic action of LB-46, H56/28 and propranolol in a concentration of 10^{-6} against isoprenaline. The dotted lines represent the curves to be expected if isoprenaline in such concentrations was applied.

P : Propranolol

H : H56/28

L : LB-46

Ordinate : % Relaxation (y)

Abscissa : Log concentration of isoprenaline (Log x)

FIG. 4. Log concentration-response curves of the antagonistic action of LB-46 in concentrations of 10^{-7} , 10^{-6} and 10^{-5} against adrenaline. The dotted lines represent the curves to be expected if adrenaline in such concentrations was applied.

The pretreatment with LB-46 in the above-mentioned concentrations caused a parallel shift of the log concentration-response curves for isoprenaline to the right without any decrease in the maximal effect.

Fig. 3 illustrates the antagonistic potency of LB-46, H56/28 and propranolol in the concentration of 10^{-6} .

The ratio of the antagonistic potency of propranolol, H56/28 and LB-46 was found to be about 1:1.5:2.2 from the slope of curves or ED_{50} for each of three drug. LB-46 exerted the most strongest antagonistic action.

2. Antagonistic action of LB-46 against adrenaline

Fig. 4 illustrates the antagonistic potency of LB-46 in concentrations of 10^{-7} , 10^{-6} and 10^{-5} against adrenaline.

LB-46 in those concentrations caused a parallel shift of the log concentration-response curves for adrenaline to the right.

The pretreatment with LB-46 in some concentrations caused either relaxation or constriction of the tracheal muscle according to a concentration of the agonist; an application of higher concentrations than about 3.2×10^{-7} of adrenaline after the complete suppression of relaxation by the pretreatment with a definite concentration of LB-46 resulted in constriction of the trachea.

The value (%) of constriction shown in Figs. 4-7 was calculated from the value of the maximal relaxing effect (100%) in the same preparation.

The maximal constricting effect was obtained after the treatment with adrenaline in

FIG. 5. Log concentration-response curves of the antagonistic action of LB-46, H56/28 and propranolol in a concentration of 10^{-6} against adrenaline. The dotted lines represent the curves to be expected if adrenaline in such concentrations was applied.

P : Propranolol

H : H56/28

L : LB-46

Ordinate : 1) % Relaxation (y)

2) % Constriction to the maximum relaxation in the same preparation (y')

Abscissa : Log concentration of adrenaline (Log x)

FIG. 6. Log concentration-response curves of the antagonistic action of LB-46 in concentrations of 10^{-7} , 10^{-6} and 10^{-5} against noradrenaline. The dotted lines represent the curves to be expected if noradrenaline in such concentrations was applied.

Ordinate : % Relaxation

Abscissa : Log concentration of noradrenaline (Log x)

higher concentrations than 10^{-5} . But the pretreatment with dibenamine of 10^{-6} suppressed this constricting effect completely.

Fig. 5 illustrates the antagonistic potency of LB-46, H56/28 and propranolol in the given concentration of 10^{-6} . Of three agents found to antagonize the relaxing effect of adrenaline, LB-46 was most potent. The ratio of the antagonistic potency of propranolol, H56/28 and LB-46 was similar to that in the experiment with isoprenaline.

3. Antagonistic potency of LB-46 against noradrenaline

Fig. 6 illustrates the antagonistic potency of LB-46 in concentrations of 10^{-7} , 10^{-6} and 10^{-5} against noradrenaline.

The pretreatment with LB-46 in those concentrations caused a parallel shift of the log concentration-response curves for noradrenaline to the right. But noradrenaline was blocked to a lesser degree than isoprenaline and adrenaline by the same concentration of LB-46.

On the other hand, the pretreatment with LB-46 caused either relaxation or constriction of the tracheal muscle according to a concentration of the agonist; an application of noradrenaline in higher concentrations above 5.6×10^{-7} after the complete suppression of relaxation by the pretreatment with LB-46 resulted in constriction of the tracheal muscle. The maximal constricting effect was obtained by the application of noradrenaline in a concentration of 10^{-5} .

Fig. 7 illustrates the antagonistic potency of LB-46, H56/28 and propranolol in the given concentration (10^{-6}) against noradrenaline.

LB-46 produced the most strongest antagonistic effect. The ratio of the antagonistic potency of three *beta*-adrenergic blocking agents was similar to that in the experiment with isoprenaline and adrenaline.

4. The spastic effect of LB-46 on tracheal muscle

With the test on the tracheal muscle of guinea pigs, a many of *beta*-adrenergic blocking agents such as pronethalol, propranolol, methoxamine, H56/28 and others were found to possess the intrinsic stimulating action in relatively higher concentrations.

Therefore, the intrinsic action of LB-46 was investigated as the preliminary experiments. A simple application of LB-46 in higher concentrations than 10^{-4} caused a spastic response of the tracheal muscles, and this stimulating effect was not so intensive as that of above-mentioned *beta*-adrenergic blocking agents.

But when no recovery from relaxation occurred after washing, the agonist-treated preparation caused a constricting effect in lower concentrations than 10^{-4} .

DISCUSSION

Ahlquist's hypothesis (7) on the concept of the adrenergic receptive mechanism tries to classify the action of catecholamines according to the ability of activating either *alpha*- or *beta*-adrenergic receptors or both.

This concept was supported by a many of studies on *alpha*-adrenergic blocking agents such as phenoxybenzamine and dibenamine to block the *alpha*-receptor activating effect, and on *beta*-adrenergic blocking agents such as DCI and propranolol to block the *beta*-adrenergic activating effect.

The previous report (8) described the *beta*-adrenergic activity of pronethalol, propranolol, H56/28 and methoxamine in the isolated tracheal muscle of guinea pigs. It revealed that H56/28 and propranolol were more potent than the other *beta*-adrenergic blocking agents and that H56/28 was qualitatively similar to, but more potent than propranolol.

FIG. 7. Log concentration-response curves of the antagonistic action of LB-46, H56/28 and propranolol in a concentration of 10^{-6} against noradrenaline. The dotted lines represent the curves to be expected if adrenaline in such concentrations was applied.

P : Propranolol

H : H56/28

L : LB-46

Ordinate : 1) % Relaxation (y)

2) % Constriction to the maximum relaxation in the same preparation (y')

Abscissa : Log concentration of noradrenaline (Log x)

In the present study the *beta*-adrenergic blocking activity of LB-46, a new *beta*-adrenergic blocking agent, was compared with that of H56/28 and propranolol in the tracheal muscle of guinea pigs.

First, when analysis was made of the concentration-response curves established for the antagonistic action of LB-46 against isoprenaline, adrenaline and noradrenaline in two different ways of plotting, the pretreatment with LB-46 caused a parallel shift of the concentration-response curves for the agonists to the right, and these curves indicated a blockade of the competitive type, as with the other *beta*-adrenergic blocking agents. That is, the mechanism of action of three agents on a *beta*-adrenergic receptor were identical, but there was a difference between the antagonistic potency of LB-46 and that of the rest. The ratio of the antagonistic potency of propranolol, H56/28 and LB-46 was found to be about 1: 1.5: 2.2. It has recently been reported that in the experiments with the sinoatrial node the antagonistic potency of these *beta*-adrenergic blocking agents is in order of LB-46 > H56/28 > propranolol [Ohkuda *et al.* (9)].

Noradrenaline was blocked to a lesser degree than both isoprenaline and adrenaline by the same concentration of LB-46: in all the preparations a given concentration of LB-46 antagonized isoprenaline and adrenaline more markedly than noradrenaline. Foster (10) observed similar facts as to the effect of propranolol on the tracheal muscle of guinea pigs. He stated that propranolol might reduce the uptake of noradrenaline into tissue stores. Prior to his report, Iversen and Whitby (11), and Iversen (12) reported that the uptake of noradrenaline into tissue stores is greater than that of adrenaline. Iversen showed that DCI, and pronethalol reduce the uptake of noradrenaline into the rat isolated heart. Foster supposed that the *beta*-adrenergic blocking agents as DCI, pronethalol and propranolol block the uptake of noradrenaline into tissue stores, that this is the basis for the differential antagonism of *beta*-blocking agents towards the catecholamines, and that the catecholamines may be all blocked to the same extent at the receptor level: but that at the same time noradrenaline may be potentiated and this potentiation may be "hidden" but serve to reduce the degree of antagonism experimentally observed.

LB-46 and propranolol seem to possess a similar action, irrespective of the validity of his assumption.

The previous paper (13) reported that an application of adrenaline or noradrenaline after the treatment with H56/28 or propranolol in relatively high doses constricted the tracheal muscle. In the present work, an application of adrenaline or noradrenaline after the treatment with LB-46 in relatively lower concentrations than with H56/28 and propranolol caused a constrictive response in the tracheal muscle. This finding confirmed the evidence that LB-46 is more potent than the other two.

The constrictive response could be inhibited by the treatment with dibenamine in adequate concentrations. Therefore, it might be due to stimulation of the *alpha*-receptor. Since the relaxing effect of adrenaline or noradrenaline usually predominates over the constricting effect, a simple application of either of the drugs appears to result in relaxation only, i.e., in the *beta*-receptor activity. But it seems likely that the treatment with a highly

potent *beta*-adrenergic blocking substance, such as LB-46, facilitates an appearance of the hidden *alpha*-adrenergic response with the catecholamines.

This constricting response by adrenaline and noradrenaline was not recognized in Foster's experiments with the tracheal muscle treated with DCI, pronethalol and propranolol. But Castro *et al.* (14) reported an induction of bronchoconstriction in the *in vivo* experiments with dogs on one hand, and on the other hand, Takagi *et al.* (15) observed the constricting response in the isolated trachea of guinea pigs by using pronethalol and propranolol.

In the present work this constricting response was observed after the treatment with such a highly potent *beta*-adrenergic blocking agent as LB-46.

SUMMARY

The relative potency of *beta*-adrenergic blocking activity of LB-46, a new *beta*-adrenergic receptor blocking agent, was compared with that of H56/28 and propranolol in the guinea pig tracheal muscle by means of the manometric method.

The interaction of LB-46 against isoprenaline, adrenaline and noradrenaline was competitive in type, as that of other *beta*-adrenergic blocking agents, and similar in nature to that of H56/28 and propranolol. But it was more potent than the latter two.

Therefore, it seems probable that the pretreatment with a highly potent *beta*-adrenergic blocking agent such as LB-46 facilitates an appearance of the hidden *alpha*-adrenergic response with the catecholamines.

REFERENCES

- 1) BLACK, J.W. AND STEPHENSON, J.S.: *Lancet* **2**, 311 (1962)
- 2) BLACK, J.W., CROWTHER, A.F., SHANKS, R.G. AND DORNHORST, A.C.: *Lancet* **1**, 1080 (1964)
- 3) ÅBLAD, B., BROGÅRD, M. AND EK, L.: *Acta pharmac. tox.* **25**, Suppl. 2, 9 (1967)
- 4) WEIDMANN, H.: *Pharmacology of LB-46 from Sandoz Laboratory Information*, April, 15 (1967)
- 5) JAMIESON, D.: *Br. J. Pharmac. Chemother.* **19**, 286 (1962)
- 6) MATSUMOTO, H.: *Seitai-no-kagaku* **6**, 113 (1954)
- 7) AHLQUIST, R.P.: *Am. J. Physiol.* **153**, 586 (1948)
- 8) OTORII, T.: *Jap. J. Smooth Muscle Res.* **5**, 76 (1969)
- 9) OHKUDA, K., SANO, N., MATSUMURA, S., CHIBA, S. AND HASHIMOTO, K.: *Folia pharmac. jap.* **65**, 27§ (1969)
- 10) FOSTER, R.M.: *J. Pharm. Pharmac.* **18**, 1 (1966)
- 11) IVERSEN, L.L. AND WHITBY, L.G.: *Br. J. Pharmac. Chemother.* **19**, 355 (1962)
- 12) IVERSEN, L.L.: *Br. J. Pharmac. Chemother.* **24**, 387 (1965)
- 13) OTORII, T.: *Folia pharmac. jap.* (*in press*)
- 14) CASTRO DE LA MATA, R., PENNA, M. AND AVIADO, D.M.: *J. Pharmac. exp. Ther.* **135**, 197 (1962)
- 15) TAKAGI, K., TAKAYANAGI, I., TAGA, F. AND OSADA, E.: *Folia pharmac. jap.* **63**, 100§ (1967)