THE $\beta$-ADRENERGIC RESPONSES IN ISOLATED SPLENIC CAPSULE AREA OF SEVERAL SPECIES OF ANIMALS

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Abstract—In isolated splenic capsules of mouse, rat, guinea pig, rabbit, cat and dog, either a single or a cumulative dose of L- and dl-isoproterenol at a concentration higher than $10^{-9}$ M, induced a relaxation which was inhibited or blocked with pretreatment of $10^{-5}$ M propranolol. At a concentration of $5 \times 10^{-6}$ M to $10^{-5}$ M L- and dl-isoproterenol, an inverse contraction was induced, and such response was prevented or reversed to one of relaxation in the presence of $10^{-5}$ M phentolamine. Application of $10^{-5}$ M epinephrine produced a strong contraction and such was reversed to one of relaxation after addition of $10^{-4}$ M phentolamine. This relaxation was reduced or blocked with pretreatment of $10^{-5}$ M propranolol. Thus, it was confirmed that both $\alpha$- and $\beta$-receptors exist in the splenic capsule area of these different species.

Catecholamines induce contraction on isolated or in vivo spleen tissues including smooth muscle areas of vessels and the capsule from various species (1–8). This contraction was identified to be the response from the stimulation of $\alpha$-adrenergic receptors.

Isoproterenol evokes a small and unclarified inhibitory effect on isolated spleen of cat (9) and an apparent relaxation of the entire spleen of mouse (10). Ignarro and Titus (10) confirmed the existence of $\beta$-receptors in mouse spleen only, and assumed there were no $\beta$-receptors in the spleen of other species such as rat, guinea pig, rabbit and monkey. Kizaki and Abiko (11) and Takano (12) also reported the lack of $\beta$-receptors in isolated spleens of kid, cat, rabbit and dog.

On the other hand, it was reported that the $\beta$-receptors did (13–15) or did not (16) exist in the vascular bed in the spleen. In addition, the conclusion was that $\beta$-receptors were to be found in the blood vessel area but not in other areas of splenic smooth muscle in the spleen of cat, in vivo (15), or that $\beta$-receptors may also exist in the splenic capsule area (13) in perfused spleen of dog.

We isolated spleen preparations of rats, mice, guinea pigs, rabbits, cats and dogs, and investigated the existence of $\beta$-receptors in splenic capsule areas in these species.

MATERIALS AND METHODS

Mice (ICR, 24 to 45 g), rats (Wistar, 170 to 380 g), guinea pigs (Hartley, 250 to 410 g), rabbits (2 to 2.5 kg), cats (2 to 3 kg) and mongrel dogs (8 to 10 kg) were used. The mice, rats and guinea pigs were sacrificed by a blow on the head, and rabbits, cats and dogs were anesthetized with pentobarbital sodium (Abbott), and a laparotomy was done. The spleen was isolated and the capsule preparation was made by sectioning the tissue at the hilus side.
Connective tissue and vessels were removed. A preparation approx. 1.5 cm in length, sometimes longer in rabbits, cats and dogs, was suspended in a circulating organ bath containing 30 ml of Locke-Ringer solution \([\text{NaCl} \ 9.0, \text{KCl} \ 0.42, \text{CaCl}_2 \ 0.24, \text{NaHCO}_3 \ 0.5, \text{glucose} \ 1.0 \ (\text{g/l})]\) at 37°C gassed with a mixture of 95% O\(_2\) and 5% CO\(_2\). Drugs were diluted with Locke-Ringer solution, and the doses were determined as a final concentration in the organ bath.

Alteration in tension was isotonically traced on a polygraph (Nihon Kohden RM-25) by means of an isotonic transducer (Nihon Kohden TD-112S). Suspending tensions were 0.5 g for mouse, 1 g for rat, guinea pig and rabbit and 1.5 g for cat and dog. The preparation was incubated in an organ bath for 60 to 120 min until the baseline had stabilized before the drug administration.

The cumulative dose-response curve with isoproterenol application was measured every 4 min by increasing the concentration of the drug without washout of the preparation. The interval between single and cumulative administrations was 60 min and adrenergic blocking agents were introduced 5 min prior to the adrenergic stimulants.

The drugs used were as follows: 1- and dl-isoproterenol hydrochloride (Nikken), propranolol hydrochloride (ICI), epinephrine hydrochloride (Sankyo), phentolamine mesylate (CIBA).

RESULTS

I] Rat

Fig. 1 demonstrates the dose-response curves with single doses of 1- and dl-isoproterenol (10\(^{-8}\) M to 10\(^{-5}\) M) before and after 10\(^{-5}\) M propranolol in the mean values of 4 to 5 experiments.

When 1- or dl-isoproterenol in a dose of less than 10\(^{-9}\) M was administered, there was no change in the tension of the preparation.

Isoproterenol (10\(^{-8}\) M to 10\(^{-5}\) M) provoked marked relaxation, and the relaxation to 10\(^{-5}\) M isoproterenol was usually slightly weaker than that to 10\(^{-6}\) M isoproterenol. As the concentration increased to 5 \times 10\(^{-5}\) M or 10\(^{-4}\) M, a contraction occurred. The contraction was frequently potentiated after 10\(^{-5}\) M propranolol treatment and reversed to one of relaxation after 10\(^{-5}\) M or 10\(^{-4}\) M of phentolamine was added. Moreover, relaxations

![Fig. 1. Single dose-response curves of 1- and dl-isoproterenol (ISOP) before (——, control) and after (-----) 10\(^{-5}\) M propranolol (PROP) treatment on splenic capsule preparations in rats. Ordinate: changes of response amplitude (mm) on the record. Abscissa: molar (M) concentrations of isoproterenol (ISOP). Each point represents a mean value of 4 experiments. The standard error is indicated by vertical bars.](image-url)
induced by 1- and dl-isoproterenol (10^{-8} M to 10^{-5} M) were depressed or blocked in the presence of 10^{-4} M propranolol. In most cases, the relaxation responded to 10^{-5} M dl-isoproterenol was converted to one of contraction after 10^{-5} M propranolol, while a pronounced relaxation occurred after 10^{-5} M phentolamine (Fig. 2). This same figure also shows the response to 10^{-5} M epinephrine. When 10^{-5} M epinephrine was administered, a strong contraction was obtained, and such was converted to one of relaxation after treatment of 10^{-4} M phentolamine. This relaxation was again replaced by a weaker contraction after a 60 min interval or the contraction became more evident in the presence of 10^{-5} M propanolol.

The cumulative dose-response curves to 1- and dl-isoproterenol are shown in Figs. 3 and 4.

In the experiment of cumulative administration, the drug was applied (10^{-8} M to 5 \times 10^{-5} M 1-isoproterenol and 10^{-8} M to 10^{-4} M dl-isoproterenol) every 4 min without washout of the preparation, and the sequence was allowed to continue for 30 min. No response was observed at doses less than 10^{-5} M of 1- and dl-isoproterenol. The relaxation sharply appeared with 10^{-7} M 1-isoproterenol or 10^{-6} M dl-isoproterenol. When the dose of 1- or dl-isoproterenol was increased to 10^{-5} M, contraction sometimes occurred. A distinct contraction was obtained by addition of 5 \times 10^{-5} M 1-isoproterenol or 10^{-4} M dl-isoproterenol. The relaxation induced by 1- or dl-isoproterenol at 10^{-7} M and 10^{-6} M decreased

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\[ \text{Fig. 2. Single dose-responses of 1- and dl-isoproterenol (ISOP) and epinephrine (EP) before and after propranolol (PROP) or phentolamine (PHENT) treatment on splenic capsule preparations in rats. ---: time in 5 min. On the 2nd line (di-isoproterenol) and 4th line (epinephrine) traces, 60 min intervals without blockade between 2nd and 3rd administrations.} \]
Fig. 3. Cumulative dose response curves of L- and dl-isoproterenol (ISOP) before and after propranolol (PROP) or phentolamine (PHENT) treatment on splenic capsule preparations in rats. On the lower trace, 60 min intervals without blockade.

Fig. 4. Cumulative dose-response curves of L- and dl-isoproterenol (ISOP) before (---, control) and after (-----) 10^{-5} M propranolol (PROP) treatment on splenic capsule preparations in rats. Representations are the same as those in Fig. 1.

or was blocked after 10^{-5} M propranolol treatment. The contraction to 5 \times 10^{-5} M L-isoproterenol or 10^{-4} M dl-isoproterenol remained the same as the control response after 10^{-5} M propranolol, while such contraction was inhibited or overcome to frequently cause a relaxation after 10^{-5} M phentolamine (Fig. 3). The response to isoproterenol returned to its initial condition gradually when the washout of preparation was repeated.

Fig. 4 shows the responses in the mean of 7 experiments with the cumulative dose of L- and dl-isoproterenol (10^{-8} M to 10^{-5} M) before and after treatment with 10^{-5} M propranolol.

II] Other species

In this experiment, mice, guinea pigs, rabbits, cats and dogs were also used.

Similar results as in the case of rat were obtained in mice, guinea pigs and rabbits. In the cases of cat and dog, administration of L- or dl-isoproterenol (10^{-6} M to 10^{-5} M) resulted in a most prominent relaxation, and when over 5 \times 10^{-5} M was given, a clear contraction was noted. As to the cumulative doses, 10^{-7} M to 10^{-6} M isoproterenol elicited a distinct relaxation in most cases, but the response to 10^{-5} M was rather weak. Contraction occurred when the drug concentration was increased from 5 \times 10^{-6} M to 10^{-5} M. After treatment with 10^{-5} M propranolol, the relaxation to L- or dl-isoproterenol in either a single or a cumulative dose was depressed or abolished, and in a few cases, the relaxation to 10^{-6} M isoproterenol was replaced by one of contraction after 10^{-5} M propranolol.
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**FIG. 5.** Upper record: single dose-responses of $10^{-7}$ M 1-isoproterenol (ISOP) before and after $10^{-5}$ M propranolol (PROP) treatment on splenic capsule preparation of dog. Sixty min intervals without blockade between 2nd and 3rd administrations. Lower record: cumulative dose-responses of $10^{-8}$ M to $10^{-4}$ M 1-isoproterenol (ISOP) before and after $10^{-5}$ M phentolamine (PHENT) $10^{-5}$ M propranolol (PROP) on splenic capsule preparation of cat. \( t \) - \( t \): time in 5 min.

traction with application of isoproterenol ($5 \times 10^{-6}$ M to $5 \times 10^{-5}$ M) was replaced by a relaxation after addition of $10^{-5}$ M phentolamine. The strongest relaxation was elicited by $10^{-5}$ M or $10^{-6}$ M isoproterenol, while administration of $10^{-5}$ M of the drug produced contraction on several occasions. Relaxation effects of 1- and dl-isoproterenol were partially overcome or abolished by $10^{-5}$ M propranolol. The contraction induced by doses of the drug higher than $10^{-5}$ M were reversed to a response of relaxation when pretreatment with $10^{-5}$ M phentolamine was given (Fig. 5).

In above mentioned results, the relaxant effects of 1- and dl-isoproterenol were clearly identified even in the isolated splenic capsule areas, thus demonstrating the existence of \( \beta \)-receptor in these species.

**DISCUSSION**

Many researchers had stated that there are \( \alpha \)-receptors in splenic smooth muscle, especially blood vessels, of species such as cat and dog, and the muscle elicits a contraction with the stimulation of the receptor (1-8). Studies of the existence of \( \beta \)-receptor in the spleen were originated by Bickerton (9) who found that when a contraction was elicited with catecholamines, addition of isoproterenol reversed it to one of relaxation and such was attributed to the stimulation of adrenergic inhibitory receptor. This relaxation produced by isoproterenol diminished or reverted to one of contraction in the presence of dichloroisoproterenol. However, the isoproterenol induced relaxation in his experiments was modest, small and not definite. Later, Ignarro and Titus (10) reported that an appreciable relaxation was observed in isolated entire spleen of mouse exposed to dl-isoproterenol and that such was inhibited or blocked by pretreatment of MJ 1999, thus indicating the existence of \( \beta \)-receptors. However, they concluded that no \( \beta \)-receptor existed in the spleen of the other species such as rat, guinea pig, cat, rabbit and monkey. Additionally, Kizaki and Abiko (11) and Takano (12) stated that the isolated spleen of kid, cat and rabbit have no
β-receptors. Nevertheless, in their experiments, the contraction elicited with epinephrine or with a high concentration of isoproterenol was enhanced by pretreatment with propranolol.

The above mentioned studies were performed in the spleen in vivo or in the isolated entire spleen, thus results obtained were the total responses including those of the vessels, the capsule and the other tissues. Though there is a disagreement in dog (16), the existence of β-receptor was suggested in the splenic vessels of cat (14) and also in other areas of splenic smooth muscle (15). Moreman et al. (13) assumed that in dog spleen, the distribution of β-receptor is preferential in the vascular area of the spleen rather than in the trabecular and capsular areas. The experimental methods regarding the β-receptor in the spleen varied with the author, and different species no doubt produce different results. The responses obtained to the entire spleen preparation could not be compared with those to the part of tissue, especially the capsular area, which responds clearly to isoproterenol. In this regard, we attempted to elicit responses of smooth muscle of the capsular area (a thick fibromuscular tissue covered by serosal-endothelium), and used a preparation in which other tissues such as large branches of splenic vessels and the splenic parenchyma or pulp were cut off. Therefore, the adrenergic responses of the smooth muscle of capsular area were probably observed.

Additionally, Ignarro and Titus (10) applied dl-isoproterenol only, while we used l- and dl-isoproterenol. No particular difference in the response was evident. In this experiment, single or cumulative administration of both l- and dl-isoproterenol enabled the splenic capsule to respond with a relaxation and species differences were noted. The degree of relaxation appeared to vary with the size of the preparation as the thinner preparations of guinea pig and smaller preparations of mouse showed weaker relaxations as compared to larger preparations from rabbit, cat and dog.

Pretreatment with propranolol (an adrenergic β-blocker) suppressed or prevented the relaxant effect of isoproterenol. On the other hand, addition of appropriate higher doses (over $10^{-5}$ M) of isoproterenol induced contraction which was inhibited or blocked by phentolamine (an adrenergic α-blocker). According to the observation with lower doses ($10^{-7}$ M to $10^{-5}$ M) of isoproterenol, relaxation appeared and was depressed or abolished by propranolol. The application of an adequate dose of epinephrine (possessing adrenergic α- and β-responses) produced a contraction and inversely induced a relaxation after phentolamine. This relaxation was diminished by pretreatment with propranolol.

Thus β-receptors exist along with α-receptors in the area of splenic capsule of rat, guinea pig, rabbit, cat and dog, and stimulation of β-receptors induces relaxation while stimulation of α-receptors induces contraction.

REFERENCES

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