Changes in the Effects of Clonidine on Left Atrium and Hindlimb Vasculature of Rats in Various Thyroid States

A Study of the Responsiveness of $\alpha_2$-Adrenoceptors in the Cardiovascular System

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SUMMARY

Postsynaptic $\alpha_2$-adrenoceptors have been reported to exist in various tissues, including vascular smooth muscle. In order to investigate the possibility of their mediating a positive inotropic change and clarify the influence of the thyroid on their responsiveness, we examined the effects of clonidine, a known $\alpha_2$-agonist, on the isolated left atria and femoral vascular beds of rats which were made hypo-, hyper- or euthyroid. Clonidine caused a dose-dependent positive inotropic change in the hypothyroid rat atrium, which was thought to be due to its $\alpha_1$-stimulating action because of the antagonistic effect exerted by either phentolamine ($10^{-6}$M) or prazosin ($10^{-7}$M), but not by yohimbine ($10^{-7}$M) or cimetidine ($10^{-5}$M). In the hyperthyroid rat atrium, clonidine exerted a negative inotropic effect at high concentrations, which was thought to be due to its direct action on the cardiac muscle. Clonidine did not cause any inotropic change in the euthyroid rat atrium. Thus, an inotropic change mediated by the postsynaptic $\alpha_2$-adrenoceptors could not be demonstrated in the rat heart. In the experiment involving hindlimb perfusion, clonidine caused vasoconstriction which was antagonized by yohimbine (10 $\mu$g/min). This effect was significantly augmented in the hypothyroid rats but not changed in the hyperthyroid ones. The vasoconstrictive effect of phenylephrine was found to be reduced in both hypo- and hyperthyroid rats. These results suggest that, in the peripheral vascular system,
thyroid function also influences the postsynaptic $\alpha_2$-adrenoceptors, but not in the same way as it affects the $\alpha_1$-adrenoceptors.

**Additional Indexing Words:**
- Postsynaptic $\alpha_2$-adrenoceptors
- Clonidine
- Vasoconstriction
- Inotropic change
- Thyroid function

It is currently accepted that $\alpha$-adrenoceptors on the nerve endings and effector organs regulate the release of catecholamine from nerve endings or evoke responses of the effector organs. These adrenoceptors are different in nature and divided into 2 subtypes, namely $\alpha_1$ for postsynaptic and $\alpha_2$ for presynaptic membranes. Recently, it has been reported that $\alpha_2$-adrenoceptors are also located on the postsynaptic membranes of various tissues, including vascular smooth muscle.

$\alpha_2$-adrenoceptors have been demonstrated in animal hearts on the basis of the negative chronotropic effect of clonidine on the accelerated heart rate induced by stimulation of the cardioaccelerator nerves. Radioligand binding assay studies have also suggested the existence of $\alpha_2$-adrenoceptors in the rat heart. However, it remains to be elucidated whether $\alpha_2$-adrenoceptors are also located postsynaptically in the heart as they are in the peripheral vascular system.

It is well known that the thyroid state affects the responsiveness of $\alpha_1$- and $\beta$-adrenoceptors in the heart. The influence of the thyroid on the vascular responses mediated by these adrenoceptors has also been studied. However, it has not yet been clarified how thyroid function affects the $\alpha_1$-mediated responses in the cardiovascular system. If postsynaptic $\alpha_2$-adrenoceptors were present in the heart and affected by the thyroid in the same way as $\alpha_1$-adrenoceptors are, the positive inotropism mediated by these adrenoceptors would presumably be more manifest in the hypothyroid state.

The present investigation was undertaken in order to 1) investigate the possibility of the postsynaptic $\alpha_2$-adrenoceptors mediating a positive inotropic change and 2) clarify the influence of thyroid function on their responsiveness. For this purpose, the effects of clonidine, a known $\alpha_2$-agonist, on the left atrium and hindlimb vasculature were examined in rats which had been made hypo-, hyper-, or euthyroid.

**Materials and Methods**

*Treatment of rats*

Young male Wistar rats (100–120 Gm) were made hypothyroid by feeding them a 0.15% 6-propyl-2-thiouracil diet over 3 months. These rats de-
veloped dryness of fur, considerable growth retardation and prominent struma. Their rectal temperatures were significantly lowered. The hyperthyroid state was induced in male Wistar rats (300–350 Gm) by subcutaneous injection of 1 mg/Kg/day of 3, 3', 5-triiodo-1-thyronine for 1 to 2 weeks. In this group of rats, the body weights were reduced and the rectal temperatures were significantly elevated. Male Wistar rats of about the same age were used as euthyroid rats.

**Experiment on left atrium**

Each rat was killed by a blow on the head, following which the heart was rapidly excised and the left atrium dissected free. The isolated left atrium was suspended in an 18 ml organ bath containing Krebs-Henseleit solution of the following composition (mM): NaCl 120; KCl 4.7; MgSO₄ 1.2; CaCl₂ 2.0; KH₂PO₄ 1.2; NaHCO₃ 25.0; glucose 14.0. The solution was maintained at a temperature of 32±0.2°C and bubbled with 95% O₂ and 5% CO₂. The left atrium was driven at a frequency of 2.5 Hz through an electrode by a square wave pulse of 3 msec duration and a voltage of approximately twice threshold. The left atrium was stretched with a resting tension of 0.5 Gm and the tension developed was recorded via a force-displacement transducer (Nihon Kohden, SB-1TH) on an ink-writing oscillograph (Nihon Kohden, RJG-4024). After equilibrating the tissue in the Krebs-Henseleit solution for about 60 min, cumulative dose-response curves were determined. After administration of the maximal effective dose, tissues were washed frequently for about 60 min. Antagonists were allowed to interact with the tissue for about 30 min before the administration of agonists. The inotropic changes were expressed as percent changes of the basal tension.

**Experiment on femoral vascular beds**

The vascular responses were examined in the femoral vascular bed perfusing the right hindlimb at a fixed flow rate with a mixture of an equal volume of Krebs-Henseleit solution and heparinized blood of male Wistar rats (500–600 Gm) by the aid of a peristaltic pump (ATTO, SJ-1211). The perfusate was oxygenated with 95% O₂ and 5% CO₂ in a silicon flask kept in a bath which was maintained at 37°C by a thermometer (Toyo, Lab-thermo LH-1000E). Rats were prepared for the hindlimb perfusion as described by Sakai. After anesthetizing rats with sodium pentobarbital (30 mg/Kg i.p.), systemic heparinization and midline incision of the abdominal wall were conducted and the right femoral artery and vein were carefully dissected. Both vessels were cannulated and the right hindlimb was completely isolated from the body. The perfusate was infused into the femoral artery and allowed to drain from the vein into another flask. The perfusion pressure
was set at approximately 100 mmHg, and about 30 min were allowed for equilibration before drugs were administered. The vascular responses were estimated from the changes in the perfusion pressure, which was continuously recorded with a pressure transducer (Toyo, MPU-0.5-290-0-III). Dose-response curves were determined by administering drugs through the arterial cannula in logarithmically increasing doses, each given after the perfusion pressure returned nearly to the pre-injection level.

Drugs

The following drugs were used: clonidine hydrochloride (C. H. Boehringer Sohn), phentolamine mesylate (Regitine, CIBA-Geigy), atropine sulfate (Merck), yohimbine hydrochloride (Katayama Chemical), cimetidine hydrochloride (Fujiwara), prazosin hydrochloride (Pfizer), 6-prophyl-2-thiouracil (Tokyo Kasei), 1-phenylephrine hydrochloride and 3, 3', 5-triiodo-1-thyronine sodium (Sigma). Drugs were dissolved before use in 0.9% saline.

Statistics

Results are expressed as the mean ± S.E. Analysis of data for significance was performed by means of paired or unpaired t-test.

Results

Effects of clonidine on the left atrial tension

The basal atrial tension was 138.0 ± 22.3, 230.4 ± 25.1 and 210.5 ± 31.7 mg in the hypo-, hyper- and euthyroid rats, respectively. The atria of the hypothyroid rats developed less tension than those of the hyper- or euthyroid rats.

Clonidine caused a positive inotropic change in the hypothyroid rats and a negative inotropic change in the hyperthyroid rats. Tracings of typical responses are presented in Fig. 1. The dose-response curves for the inotropic effect of clonidine are given in Fig. 2. In the hypothyroid rats, clonidine

![Diagram](image)

Fig. 1. Typical positive and negative inotropic effects of clonidine in the left atrium of hypothyroid (A) and hyperthyroid (B) rat. (a) control, (b) $10^{-9}$ M, (c) $3 \times 10^{-7}$ M, (d) $10^{-8}$ M, (e) $3 \times 10^{-8}$ M, (f) $10^{-7}$ M, (g) $10^{-4}$ M.
Fig. 2. Effects of clonidine on left atrial tension. ● ● : hypothyroid rats (n=18-22), × × × × : hyperthyroid rats (n=15-19), ○ ○ ○ ○ : euthyroid rats (n=4-6). Numbers in parentheses indicate the values in additional experiments. Significant difference from the respective value in the euthyroid rats: *p<0.05, **p<0.01, ***p<0.001. Values are given as the mean±S.E.

Fig. 3. Effects of various antagonists on the positive inotropic effect of clonidine in hypothyroid rats. (A) phentolamine (10^-6 M) (n=4-5), (B) prazosin (10^-7 M) (n=4-6), (C) yohimbine (10^-7 M) (n=4-5), (D) cimetidine (10^-4 M) (n=4-6). ○ ○ ○ ○ : without antagonist, ● ● ● ● : with antagonist. Values are given as the mean±S.E.
(10\(^{-7}\)–10\(^{-5}\) M) exerted a dose-dependent positive inotropic effect. The left atrial tension increased by 37.3±4.6% at maximum. The positive inotropic effect of clonidine was confirmed to be reproducible in the preliminary studies. In the hyperthyroid rats, on the other hand, clonidine (10\(^{-5}\)–10\(^{-4}\) M) produced a dose-dependent negative inotropic effect. At 10\(^{-4}\) M, clonidine decreased the tension by 41.2±5.9%. Although clonidine produced a slight negative inotropic effect in the euthyroid rats, it was not dose-related.

To clarify the mechanism by which clonidine caused the inotropic changes, the effects of various antagonists on the action of clonidine were examined. Results are summarized in Fig. 3 for the hypothyroid rats. The positive inotropic action of clonidine was effectively antagonized by phentolamine (10\(^{-6}\) M) or prazosin (10\(^{-7}\) M). The dose-response curves were shifted to the right by these drugs. Neither yohimbine (10\(^{-7}\) M) nor cimetidine (10\(^{-5}\) M) caused significant changes in the positive inotropic effect of clonidine. Thus, it was apparent that the positive inotropic change induced with clonidine was mediated by \(\alpha_1\)-adrenoceptors.

Fig. 4 shows the negative inotropic effect of clonidine in the hyperthyroid rats with and without the presence of various antagonists. Since clonidine exhibited a more prominent negative inotropic effect when the dose was repeated, the second of the dose-response curves determined with repeated dosing in the different preparations was used as the control in this
case. As shown in Fig. 4, phentolamine (10⁻⁶ M), yohimbine (10⁻⁷ M) or atropine (10⁻⁶ M) failed to antagonize the negative inotropic effect of clonidine. The negative inotropic effect of clonidine was, therefore, thought to be due to its direct action on the cardiac muscle.

**Effect of clonidine on the hindlimb vasculature**

Clonidine caused an increase in perfusion pressure in a dose-dependent manner. Typical responses are traced in Fig. 5. The magnitude of vasoconstriction induced with clonidine was increased in the hypothyroid rats. After the maximal response returned nearly to the basal level, yohimbine was infused through the cannula continuously at a rate of 10 µg/min until the end of the experiment. Continuous infusion of yohimbine was performed because of preliminary studies where the antagonistic effect of yohimbine was shown to be short-lasting. In the presence of yohimbine, the effects of clonidine were reduced as shown in Fig. 5. The vasoconstrictive effect of clonidine is summarized in Fig. 6. The increase in perfusion pressure seen with the administration of clonidine at a dose of 10⁻⁷ Gm or more was significantly augmented in hypothyroid rats. In the hyperthyroid rats, the dose-response curve for clonidine was almost the same as in the euthyroid rats. At maximum, clonidine increased the perfusion pressure by 35.5±7.4, 18.8±1.8 and 16.8±3.0 mmHg in the hypo-, hyper- and euthyroid rats, respectively. Thus, the vasoconstrictive effect of clonidine was considerably augmented in

![Fig. 5. Typical vasoconstrictive effect of clonidine and its antagonism by yohimbine (10 µg/min i.a.) in the rat femoral vascular bed. (A) upper traces, hypothyroid rat, (B) middle traces, hyperthyroid rat, (C) lower traces, euthyroid rat.](image-url)
hypothyroid rats. Following treatment with yohimbine (10 µg/min), the dose-response curves were shifted to the right in each thyroid state.

**Effect of phenylephrine on the hindlimb vasculature**

The vasoconstrictive effect of phenylephrine was examined and compared with that of clonidine. Phenylephrine caused a dose-dependent increase in perfusion pressure. Typical responses are demonstrated in Fig. 7. The responses induced with phenylephrine were different in mode from those brought about by clonidine, the rate of onset as well as recovery being fast in the former and relatively slow in the latter. As shown in Fig. 7, the magnitude of vasoconstriction induced with phenylephrine was smaller in both hypo- and hyperthyroid rats than in euthyroid rats. After the maximal response returned nearly to the basal level, phentolamine (3 µg) was injected through the cannula by bolus. After the phentolamine injection, the vasoconstriction seen with phenylephrine decreased in magnitude. In Fig. 8 the vasoconstrictive effect of phenylephrine is summarized. The increase in perfusion pressure with phenylephrine was reduced in both hypo- and hyperthyroid rats. The reduction was statistically significant at the dose of 10⁻⁶ Gm in the hypothyroid rats and at all doses tested in the hyperthyroid ones. The maximum increase in perfusion pressure attained by phenylephrine was 142.3±22.1, 111.4±13.6 and 188.5±31.3 mmHg in the hypo-, hyper- and
Typical vasoconstrictive effect of phenylephrine and its antagonism by phentolamine (3 μg i.a.) in the rat femoral vascular bed. (A) upper traces, hypothyroid rat, (B) middle traces, hyperthyroid rat, (C) lower traces, euthyroid rat.

euthyroid rats, respectively. From these results, it was evident that phenylephrine was less vasoconstrictive in hypo- and hyperthyroid rats than in euthyroid rats. Phentolamine (3 μg i.a.) effectively antagonized the vasoconstrictive action of phenylephrine in each thyroid state.

DISCUSSION

Clonidine produced a positive inotropic effect in the hypothyroid rats and a negative inotropic effect in the hyperthyroid rats (Figs. 1, 2). The positive inotropic effect of clonidine was antagonized by both phentolamine (10^{-6} M) and prazosin (10^{-7} M), but not influenced by either yohimbine (10^{-7} M) or cimetidine (10^{-5} M) (Fig. 3). Consequently, in rats, the positive
inotropic effect of clonidine can be attributed to its $\alpha_1$-stimulating action. Schümann et al.\(^{10}\) reported on the $\alpha_1$-stimulating action of clonidine in rabbit papillary muscle, where clonidine competed with phenylephrine for the same receptor site. Csongrady and Kobinger\(^{11}\) reported that clonidine produced a positive inotropic effect via $H_2$-receptors in the guinea-pig heart. However, the positive inotropic effect of clonidine was not antagonized by cimetidine (10^{-5} M) in the present study (Fig. 3).

In the hyperthyroid rats, clonidine exerted a negative inotropic effect at high concentrations that was not antagonized by phentolamine (10^{-6} M), yohimbine (10^{-7} M) or atropine (10^{-6} M) (Fig. 4). This suggests a direct action of clonidine on cardiac muscle. Washizu\(^{12}\) reported a negative inotropic effect of clonidine in the isolated guinea-pig heart at concentrations higher than 1 mM, and he ascribed this to its inhibitory effect on Ca^{2+} action.

It is well known that the responsiveness of $\alpha_1$-adrenoceptors in the heart is increased in the hypothyroid state and decreased in the hyperthyroid state.\(^{5}-7\) Therefore, it seems likely that the inotropic effect of clonidine was changed with the alteration of the responsiveness of $\alpha_1$-adrenoceptors in the hypothyroid or hyperthyroid state.

The presynaptic action of clonidine has been studied in the rabbit...
pulmonary artery, guinea-pig atrium and rat anococcygeus muscle. In these studies, the $\alpha_2$-effect of clonidine was observed at a dose of less than $10^{-8}$ M. However, in the present experiment, an inotropic change was almost never observed in any thyroid state when clonidine was administered at that concentration. Thus, inotropic change mediated by the postsynaptic $\alpha_2$-adrenoceptors was not demonstrated in the rat heart.

It is well known that $\alpha_2$-adrenoceptors are also located on the postsynaptic membranes of various tissues. One of the authors' main concerns was the influence of thyroid function on the postsynaptic $\alpha_2$-adrenoceptors in the cardiovascular system. In hypothyroid rats, the vasoconstriction seen with clonidine was significantly augmented, while in hyperthyroid rats, it was almost the same as in euthyroid rats (Fig. 6). The vasoconstriction seen with phenylephrine was suppressed both in the hypo- and hyperthyroid rats (Fig. 8). The present findings partially agree with those of Schumann et al, who reported that the effect of phenylephrine on vasoconstriction was reduced in the hypothyroid state. In their study, however, clonidine was less effective in the hypothyroid state, in contradiction to the present findings. Since the vasoconstrictive responses were estimated from the changes in the diastolic blood pressure of pithed rats in their experiment, the inconsistency of the results may be explained by the difference in the population of $\alpha_1$- and $\alpha_2$-adrenoceptors in the different vascular beds, which was suggested by Jauernig et al and De Mey and Vanhoutte.

To estimate the responsiveness of the adrenoceptors concerned, the selectivity of the given agonist and antagonist deserves close attention. In this study, the pharmacological tools for the investigation of $\alpha_2$-responsiveness were clonidine and yohimbine, both of which, unfortunately, are not sufficiently selective for $\alpha_2$-adrenoceptors; thus, it seemed inappropriate to estimate the responsiveness of $\alpha_2$-adrenoceptors directly from the responses induced with these drugs. Phenylephrine is a highly selective $\alpha_1$-agonist and was therefore thought to contribute to this investigation when compared to clonidine.

In the hypothyroid rats, since the $\alpha_1$-mediated vasoconstriction seen with phenylephrine was reduced, the augmented clonidine action may indicate that the responsiveness of $\alpha_2$-adrenoceptors is increased in the peripheral vascular system. This contrasts with the results of Gross et al, who reported that, in the brain cortex of hypothyroid rats, the sensitivity of $\alpha_2$-adrenoceptors was unchanged. In hyperthyroid rats, clonidine-induced vasoconstriction was almost the same as in euthyroid rats. Since the effect of phenylephrine (i.e., $\alpha_1$-mediated vasoconstriction) was reduced in hyperthyroid rats, the effect of clonidine may suggest an increased responsiveness of $\alpha_2$-adrenoceptors.
in the hyperthyroid state, where the pressor responses with clonidine were apparently the same as in the euthyroid state as a result of paradoxical changes in $\alpha_1$- and $\alpha_2$-mediated vasoconstriction. However, this suggestion remains speculative and further study with a more selective $\alpha_2$-agonist is needed.

From the vasoconstrictive responses induced with clonidine and phenylephrine, it was estimated that, in both the hypo- and hyperthyroid state, the overall involvement of postsynaptic $\alpha_2$-adrenoceptors in vasoconstriction is increased in relation to $\alpha_1$-adrenoceptors.

It cannot be determined, from this study, how the underlying process of $\alpha_2$-mediated vasoconstriction is affected by thyroid function. It has been demonstrated by radioligand binding assay that, in altered thyroid states, $\alpha$- as well as $\beta$-adrenoceptors are changed in number or affinity. However, data using radioligand binding assay are not available for the biochemical changes in the $\alpha_2$-adrenoceptors.

It is of pathophysiological significance to clarify the influence of the thyroid on the postsynaptic $\alpha_2$-adrenoceptors in the cardiovascular system, because it will contribute to a better understanding of the cardiovascular hemodynamics in altered thyroid states.

In conclusion, the results of the present study suggest that, in the peripheral vascular system, thyroid function also influences the postsynaptic $\alpha_2$-adrenoceptors, but not in the same way as it affects the $\alpha_1$-adrenoceptors. The responsiveness of the postsynaptic $\alpha_2$-adrenoceptors was increased in the hypothyroid state and possibly also in the hyperthyroid state, while that of $\alpha_1$-adrenoceptors was reduced in both of these thyroid states. A postsynaptic $\alpha_2$-mediated inotropic change was not demonstrated in the rat heart.

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