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# Location of $\alpha_1$-adrenoceptors relative to $\beta$-adrenoceptors in rat myocardium

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**Abstract**

Electrically driven rat papillary muscles (1 Hz) were examined for the location of their $\alpha_1$-adrenoceptor and $\beta$-adrenoceptor populations relative to each other. We determined the horizontal position of the dose-response curves for the positive inotropic effects exerted by noradrenaline in the absence and presence of the neuronal uptake blocker cocaine and in the absence and presence of the $\beta$-adrenoceptor antagonist timolol and of the $\alpha_1$-adrenoceptor antagonist prazosin. Cocaine slightly shifted the dose-response curves for $\alpha_1$-adrenoceptor stimulation to a lower concentration of agonist. In contrast, the dose-response curve for $\beta$-adrenoceptor stimulation was markedly shifted by cocaine to a lower concentration of agonist. Experiments with corticosterone (an extraneuronal uptake blocker) revealed no differential shift of either of the dose-response curves. Together, these data indicate that the $\alpha_1$-adrenoceptor population is located more distantly from the adrenergic nerve terminals than the $\beta_1$-adrenoceptor population in rat myocardium.

*Keywords:* $\alpha_1$-Adrenoceptor; $\beta$-Adrenoceptor; Localisation; Myocardium, rat; Noradrenaline; Uptake blocker

## 1. Introduction

It is well established that activation of the myocardial $\alpha_1$-adrenoceptors separately from the $\beta$-adrenoceptors will elicit a positive inotropic response in mammalian hearts (for reviews, e.g. Scholz, 1980; Brückner et al., 1985; Osnes et al., 1985; Benfey, 1993; Fedida, 1993; Terzic et al., 1993). Recent studies showed a contribution of this effect to the final inotropic response elicited by noradrenaline in the rat and the rabbit heart (Skomedal et al., 1988b, 1989). The $\alpha_1$-adrenergic inotropic component represents about 25% of the total positive inotropic response to noradrenaline in rats and the $\beta$-adrenergic component represents the final 75% (Skomedal et al., 1988b). In rat myocytes the $\beta$-adrenoceptors are apparently of the $\beta_1$ subtype (Buxton and Brunton, 1985) and this subtype is thought to be innervated, i.e. located in or close to the sympathetic synaptic cleft (Bryan et al., 1981; Stene-Larsen, 1981; Ariëns and Simonis, 1983). Thus, these $\beta_1$-adrenoceptors respond preferentially to noradrenaline released from the nerve terminals.

Little is known about the location of the $\alpha_1$-adrenoceptor population in relation to the sympathetic nerve terminals and thus also in relation to the location of the $\beta$-adrenoceptor population in cardiac myocytes. We therefore found it of interest to investigate if the different functional role of the two adrenoceptor populations would be reflected by a different location in relation to sympathetic nerve endings. Previous studies (Verity, 1971; Ebner and Waud, 1978) showed that the potentiating effect of neuronal uptake blockade upon noradrenaline is inversely influenced by the distance between a receptor population and the nerve terminals. We investigated the relative location of adrenoceptors by studying the potentiating effect of neuronal uptake blockade upon the positive inotropic response to noradrenaline when stimulating the two adrenoceptor systems separately. As noradrenaline is the best substrate for neuronal uptake (in contrast to adrenaline, phenylephrine and isoprenaline) (Iversen, 1967, 1973), this agonist was chosen for the present experiments. This experimental approach, as reviewed by Stene-Larsen (1981), was previously used to study the location in relation to sympathetic nerve endings of cardiac $\beta_1$- and $\beta_2$-adrenoceptors in different vertebrates.

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Our experiments indicate that in the rat papillary muscles, the $\alpha_1$-adrenoceptor population is located more distantly from the sympathetic nerve terminals than the $\beta_1$-adrenoceptor population.

## 2. Material and methods

### 2.1. Isolated papillary muscles

The method for isolating papillary muscles was adapted from the description given by other investigators (Henderson et al., 1969). Hearts were isolated from ether-anesthetized male Wistar albino rats weighing 180–240 g and transferred to ice-cold 0.9% NaCl. The aorta was cannulated, and the coronaries were perfused at 31° C (pH 7.4) with a medium similar to that described below apart from NaCl, which was 121.0 mmol/l, and $CaCl_2$, which was 0.5 mmol/l. During this perfusion one left papillary muscle was ligated and dissected free. It was mounted in an organ bath containing 18.0 ml of a physiological salt solution containing the following (in mmol/l): 119.0 NaCl, 3.0 KCl, 2.0 $CaCl_2$, 1.2 $MgSO_4$, 2.4 $KH_2PO_4$, 24.9 $NaHCO_3$ and 10 glucose and equilibrated with 95% $O_2$-5% $CO_2$ at 31° C (pH 7.4). Muscles were driven electrically by square-wave impulses of alternating polarity at a frequency of 1 Hz through platinum electrodes parallel to the muscles for field stimulation. The duration of the impulses was 5 ms, and the current was approximately 20% above threshold (10–20 mA), which was determined in each experiment. The isometrically contracting muscles were stretched to the maximum of the length-tension curve (final resting tension 3–5 mN). The developed tension was recorded by a Grass force-displacement transducer (model FT03C) connected to a Grass polygraph recorder model RPS7C8B equipped with 7DAG driver amplifiers, with 7P1F bridge amplifiers and with 7P20C derivators. Oscilloscope recordings were made with a Tektronix storage oscilloscope equipped with a 5A14N four-channel amplifier and a 5B10N time base unit. The muscles were allowed to equilibrate for 45 min in the salt solution mentioned above. The solution was then changed, and before the addition of agonist the muscles were equilibrated for another 15–20 min in the same solution either without or with adrenoceptor antagonists, and neuronal and extraneuronal uptake blockers as appropriate. The mechanical response in the papillary muscles at the end of the second equilibration period was taken as the control response.

### 2.2. $\alpha_1$-Adrenoceptor binding

Standard receptor-binding studies were performed with crude homogenates of rat ventricular myocardium. The ventricles were homogenized and incubated in a buffer containing 50 mmol/l Tris-HCl, 10 mmol/l $MgCl_2$, 0.1 mmol/l ascorbate and 0.4% bovine serum albumin. Incubation was carried out at 25° C and pH 7.4, for 45 min in order to obtain equilibrium conditions. [$^3$H]Prazosin (specific activity 3034.0 GBq/mmol) was used as radiolabelled ligand. The apparent $pK_D$ value determined in saturation studies was $10.01 \pm 0.12$ ($n = 3$). In the competition studies the concentration of radiolabelled ligand was about 300 pmol/l. Competing ligands were added in increasing concentrations in ranges that are relevant for the actual use in functional studies. Separation of bound and free ligands was achieved by filtration of the incubate through Whatman GF/C glass fiber filters.

### 2.3. Experimental design

The inotropic responses to separate $\alpha_1$-, $\beta$- and to combined adrenoceptor stimulation, respectively, exerted by noradrenaline were studied. Pure $\alpha_1$- and $\beta$-adrenoceptor-mediated inotropic effects of noradrenaline were obtained in the presence of appropriate receptor antagonists: the $\beta$-adrenoceptor antagonist timolol ($10^{-6}$ mol/l) and the $\alpha$-adrenoceptor antagonist prazosin ($10^{-7}$ mol/l), respectively. At these concentrations the receptor antagonists prevented the response to noradrenaline through the respective receptor systems (Aass et al., 1983; Skomedal and Osnes, 1983; Skomedal et al., 1988b). The presence of either antagonist did not influence the basal function of the muscles with respect to mechanical performance or electrical stimulation threshold. The final concentration of the neuronal uptake blocker cocaine was $3 \times 10^{-5}$ mol/l and was chosen according to Kenakin (1980). As extraneuronal uptake blocker, corticosterone was chosen according to Iversen and Salt (1970), and the final concentration was $3 \times 10^{-5}$ mol/l. These concentrations will inhibit approximately 95% of the neuronal and the extraneuronal uptake of noradrenaline, respectively. Corticosterone, dissolved in 96% ethanol, and cocaine were diluted in the salt solution, which was prewarmed and gassed for 20 min before use. The final concentration of ethanol in the organ bath did not exceed 0.3‰, which has been shown (Skomedal et al., 1988a) not to influence the function of the rat papillary muscles. A minor and short-lasting increase of the stimulation threshold of the muscle was seen when adding corticosterone to the organ bath. A slight decline in maximal developed tension was observed when both uptake blockers were present in the organ bath.

Noradrenaline was added directly to the organ bath in a cumulative way in volumes of 25 and 75 $\mu$l to give the appropriate final concentrations. Noradrenaline was completely mixed in the bath within 2–3 s.

### 2.4. Calculation and statistics

The values after drug responses were calculated as percentage of control (100%). Dose-response curves were constructed according to Ariëns and Simonis (1983), by estimating $EC_{10}$ to $EC_{100}$ for each single experiment and calculating the corresponding means. The values are given as means ± S.E.M. The significance levels of differences were expressed by calculating $P$ according to Wilcoxon's two-sample test or to the Student's $t$-test. $P$ less than or equal to 0.05 is considered to indicate statistically significant differences.

### 2.5. Drugs

Prazosin hydrochloride was kindly supplied by Pfizer (New York, NY, USA). Timolol maleate was kindly supplied by Merck, Sharp and Dohme (Rahway, NJ, USA). (−)-Noradrenaline-(+)-bitartrate, (−)-phenylephrine-hydrochloride, (±)-cocaine-hydrochloride and corticosterone-21-acetate were purchased through Norwegian Medical Depot. Stock solutions were prepared in double-distilled water and kept at −20° C to avoid oxidation. Corticosterone was prepared in 96% ethanol and kept at −20° C. Further dilutions of the drugs were made daily and kept cool (0–4° C) and dark. Repeated experiments showed that drug solutions treated in these ways were stable.

## 3. Results

### 3.1. Effect of neuronal uptake blockade by cocaine on adrenoceptor stimulation

Cumulative dose-response curves for the positive inotropic response to noradrenaline (expressed as $T'_{max}$) in the presence of timolol ($10^{-6}$ mol/l) or prazosin ($10^{-7}$ mol/l), and without adrenoceptor blockade (Fig. 1) were examined in the absence and presence of cocaine ($3 \times 10^{-5}$ mol/l).

*$\alpha_1$-Adrenoceptor stimulation by noradrenaline*

Cocaine slightly shifted the dose-response curve of $\alpha_1$-adrenoceptor stimulation (noradrenaline in the

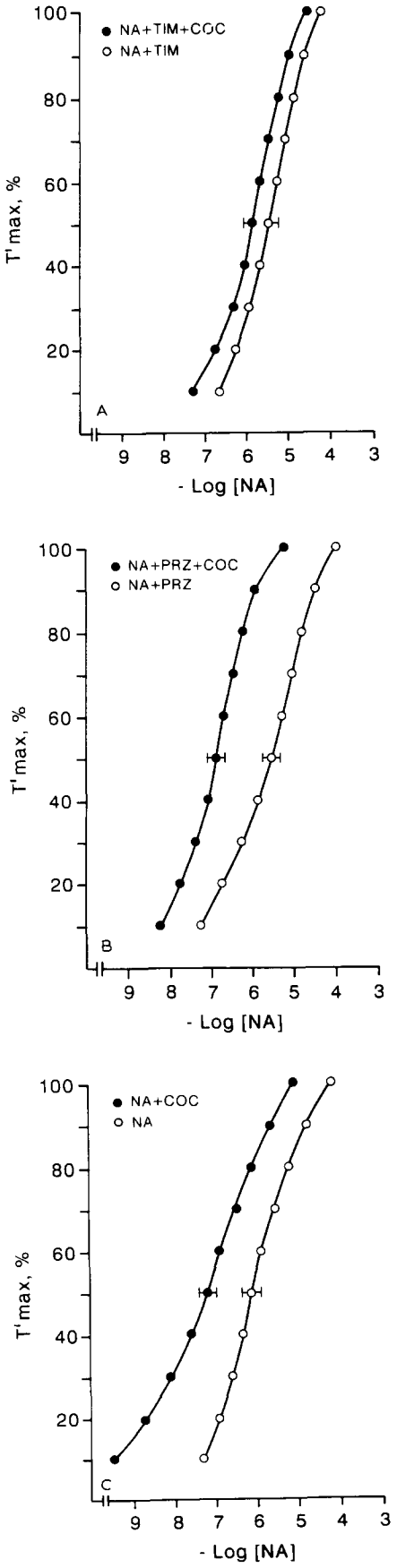


Fig. 1. Cumulative dose-response curves of noradrenaline (NA): (a) Inotropic response to NA in the presence of timolol (TIM) in the absence and presence of cocaine (COC). (b) Inotropic response to NA in the presence of prazosin (PRZ) in the absence and presence of COC. (c) Inotropic response to NA in the absence and presence of COC. The concentrations were: PRZ ($10^{-7}$ mol/l), TIM ($10^{-6}$ mol/l) and COC ($3 \times 10^{-5}$ mol/l). Abscissa: negative log of NA concentration (mol/l). Ordinate: inotropic effect of NA as a percentage of the maximal response, expressed as $T'_{max}$. Horizontal bars indicate S.E.M. of $pD_2$ ($n = 6$–8).

Table 1
Maximal inotropic responses (expressed as $T'_{max}$) to noradrenaline in the absence and presence of uptake blockers

| Adrenoceptor stimulation | Mean ± S.E.M. of max | n |
|---|---|---|
| α-Adrenergic | | |
| NA + TIM | 142.5 ± 4.5 | 7 |
| NA + TIM + COC | 143.9 ± 4.3 | 13 |
| NA + TIM + COC + CORT | 152.8 ± 5.1 | 4 |
| β-Adrenergic | | |
| NA + PRZ | 174.1 ± 4.1 | 7 |
| NA + PRZ + COC | 187.8 ± 10.6 | 10 |
| NA + PRZ + COC + CORT | 165.6 ± 7.1 | 4 |
| Combined | | |
| NA | 185.0 ± 13.4 | 7 |
| NA + COC | 196.5 ± 9.8 | 10 |
| NA + COC + CORT | 176.7 ± 12.6 | 6 |

Maximal values expressed as percentages of control values. NA = noradrenaline, TIM = $10^{-6}$ mol/l timolol, PRZ = $10^{-7}$ mol/l prazosin, COC = $3\times10^{-5}$ mol/l cocaine, CORT = $3\times10^{-5}$ mol/l corticosterone.

presence of timolol) to lower concentrations of agonist. The $pD_2$ value of $T'_{max}$ increased by 0.39 ± 0.21 log units (n.s.), corresponding to a 2- to 3-fold potentiation of the agonist (Fig. 1a). The maximal inotropic response, expressed as $T'_{max}$, was not significantly changed by cocaine (Table 1).

*β-Adrenoceptor stimulation by noradrenaline*

Cocaine significantly shifted the dose-response curve of β-adrenoceptor stimulation (noradrenaline in the presence of prazosin) to lower concentrations of agonist. The $pD_2$ value of $T'_{max}$ was increased by 1.37 ± 0.21 log units, corresponding to an about 22-fold potentiation of the agonist ($P < 0.01$, Fig. 1b). This effect was statistically significantly greater than the potentiation by cocaine of the effect of $\alpha_1$-adrenoceptor stimulation. The lusitropic response, expressed as the relaxation onset index ($T''_{min}/T'_{max}$), a specific indicator of β-adrenoceptor stimulation (Osnes et al., 1985), was also examined (Table 2). Cocaine shifted the dose-response curve for this parameter also towards lower concentrations, increasing the $pD_2$ value by 0.96 ± 0.36 log units, corresponding to a 10-fold potentiation ($P < 0.05$).

Table 2
Effect of cocaine on horizontal position of the dose-response curves for the lusitropic effect of noradrenaline (expressed as $T''_{min}/T'_{max}$)

| | $pD_2$ | $\Delta pD_2$ |
|---|---|---|
| NA + PRZ | 5.96 ± 0.16 | |
| Shift elicited by cocaine | | 0.96 ± 0.36 |
| NA | 6.12 ± 0.12 | |
| Shift elicited by cocaine | | 1.05 ± 0.31 |

NA = noradrenaline, PRZ = $10^{-7}$ mol/l prazosin. When used the concentration of cocaine was $3\times10^{-5}$ mol/l. Results are expressed as means ± S.E.M. of at least four determinations.

The maximal inotropic response, expressed as $T'_{max}$, was not significantly changed by cocaine (Table 1).

*Combined adrenoceptor stimulation by noradrenaline*

Cocaine shifted the dose-response curve of combined adrenoceptor stimulation to lower concentrations of agonist. The $pD_2$ value of $T'_{max}$ increased by 1.06 ± 0.22 log units, corresponding to an 11- to 12-fold potentiation of the agonist ($P < 0.05$, Fig. 1c). The dose-response curve for the relaxation onset index was also shifted to lower concentrations of the agonist by cocaine (Table 2). The $pD_2$ value increased by 1.05 ± 0.22 log units, corresponding to an about 11- to 12-fold potentiation of agonist ($P < 0.05$).

The maximal inotropic response, expressed as $T'_{max}$, was slightly increased by cocaine (Table 1).

### 3.2. Effects of extraneuronal uptake blockade

In order to test whether extraneuronal uptake could influence the results obtained during neuronal uptake blockade, we studied the effect of corticosterone ($3\times10^{-5}$ mol/l) on the dose-response curves of noradrenaline in the presence of cocaine.

Corticosterone shifted the dose-response curve for the inotropic effect (expressed as $T'_{max}$) to lower concentrations of agonist with an increase of the $pD_2$ value by 0.74 ± 0.23 for $\alpha_1$-adrenoceptor stimulation, by 0.53 ± 0.25 for β-adrenoceptor stimulation and by

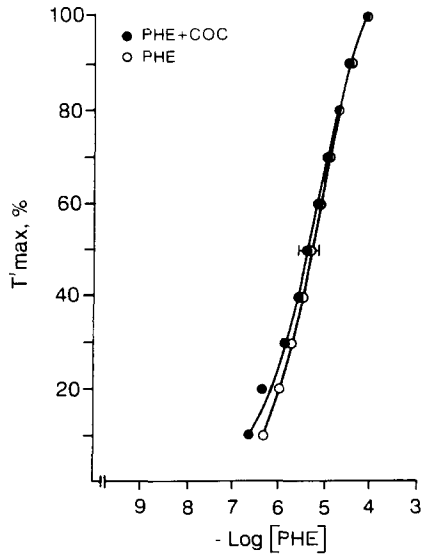


Fig. 2. Cumulative dose-response curves for the inotropic response exerted by phenylephrine (PHE) in the presence of timolol ($10^{-6}$ mol/l) in the absence and presence of cocaine (COC) ($3\times10^{-5}$ mol/l). Abscissa: negative log of PHE concentration (mol/l). Ordinate: inotropic effect of PHE as a percentage of the maximal response, expressed as $T'_{max}$. Horizontal bars indicate S.E.M. of $pD_2$ ($n = 4$).

$0.45 \pm 0.09$ for combined adrenoceptor stimulation. Corticosterone also shifted the dose-response curves for the relaxation onset index ($T''_{min}/T'_{max}$) to lower concentrations of agonist with an increase of the $pD_2$ value by $0.34 \pm 0.22$ for $\beta$-adrenoceptor stimulation and by $0.62 \pm 0.26$ for combined stimulation.

There were no statistically significant differences between the shifts of the various dose-response curves, there being a 2- to 5-fold potentiation of the agonist. Thus blockade of extraneuronal uptake by corticosterone did not differentially potentiate either dose-response curve of noradrenaline.

There were small statistically insignificant changes of the maximal inotropic responses in the presence compared to the absence of corticosterone (Table 1).

### 3.3. Interference by uptake blockers with $\alpha_1$-adrenoceptors

*Cocaine*

As some neuronal uptake blockers act as $\alpha_1$-adrenoceptor antagonists (e.g. desmethylimipramine), it was of interest to test if cocaine had a similar effect. We performed two types of control experiments: (1) A functional study with phenylephrine as an $\alpha_1$-adrenoceptor agonist (in the presence of timolol) because phenylephrine is a poor substrate for neuronal uptake compared to noradrenaline (Iversen, 1967). Cocaine did not shift the dose-response curve for phenylephrine (Fig. 2), thus indicating no functional interference by cocaine with the myocardial $\alpha_1$-adrenoceptor system. (2) In the second type of experiment we performed radioligand binding studies of $\alpha_1$-adrenoceptors with [$^3$H]prazosin. Up to a final concentration of $10^{-4}$ mol/l of cocaine there was a negligible inhibition of specifically bound [$^3$H]prazosin. Thus, the concentration of $3 \times 10^{-5}$ mol/l cocaine used in the functional studies did not interfere with $\alpha_1$-adrenoceptor binding and would thus not disturb the access of ligands to $\alpha_1$-adrenoceptors (Fig. 3).

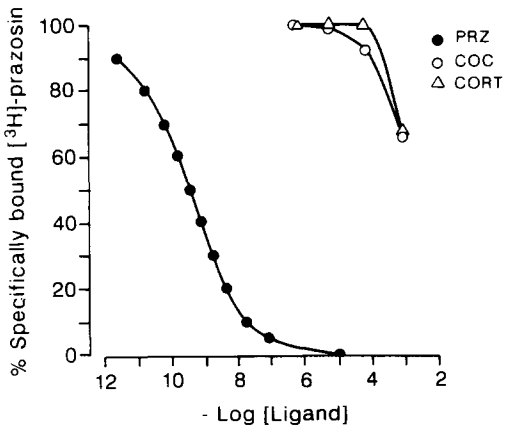


Fig. 3. Radioligand binding of [$^3$H]prazosin to $\alpha$-adrenoceptors. Effects of prazosin (PRZ), cocaine (COC) and corticosterone (CORT) on specific binding of [$^3$H]prazosin. Abscissa: negative log of concentration of unlabeled ligands (mol/l). Ordinate: Specifically bound [$^3$H]prazosin as a percentage of maximum. Results are presented as mean of 3–4 experiments.

*Corticosterone*

To test whether corticosterone would bind to the $\alpha_1$-adrenoceptors and hence possibly influence the results, we performed radioligand binding studies using [$^3$H]prazosin. Corticosterone did not inhibit specifically bound [$^3$H]prazosin up to a final concentration of $10^{-4}$ mol/l (Fig. 3). Thus, corticosterone in the concentration ($3 \times 10^{-5}$ mol/l) used in the functional studies did not interfere with the $\alpha_1$-adrenoceptor and would not influence the access of ligands to this receptor type.

## 4. Discussion

The main findings of the present study were that neuronal uptake blockade by cocaine potentiated the $\alpha_1$-adrenoceptor-mediated positive inotropic response to noradrenaline far less than the $\beta$-adrenoceptor-mediated positive inotropic response. This difference could not be explained by a differential influence on the extraneuronal uptake mechanism.

During $\beta$-adrenoceptor stimulation there was a pronounced shift of the dose-response curves towards lower concentrations of noradrenaline when cocaine was present in the organ bath. Cocaine potentiated the effects 10–20 times. From the accepted mechanisms of action of cocaine, these shifts of the dose-response curves to lower concentrations of the agonist are due to inhibition of neuronal uptake of noradrenaline and consequently increased availability of noradrenaline at the receptor site for a given concentration of noradrenaline in the organ bath.

Bevan and Verity (1967) showed in smooth muscle cells that potentiation by neuronal uptake blockade of an agonist effect mediated by a receptor population is a function of the distance between the nerve terminal and the receptor population. That is, the closer to the nerve terminal the receptor population is located, the more pronounced the potentiation will be. In these experiments this is expressed as a shift of the dose-response curves towards lower concentrations of noradrenaline (i.e. higher $pD_2$ values). The minimum distance between the nerve terminal and the receptors that gives potentiation of agonist by neuronal uptake blockade was shown to be approximately 350 Å (Trendelenburg, 1965; Bennet and Rogers, 1967; Bevan and Verity, 1967). The present data thus strongly indicate that the rat heart $\beta$-adrenoceptor population is located close to or in the synaptic cleft. This is in

agreement with the $\beta_1$-adrenoceptors being the functional subtype in rat myocytes (Buxton and Brunton, 1985).

The dose-response curve of pure $\alpha_1$-adrenoceptor stimulation in the same experimental setting was subjected to a slight shift towards lower concentrations of agonist when cocaine was present in the organ bath, corresponding to a 2- to 3-fold potentiation. This was far less than that seen during $\beta$-adrenoceptor stimulation, which was potentiated about 8 times more than during $\alpha$-adrenoceptor stimulation. Thus neuronal uptake blockade increased the availability of noradrenaline to the $\alpha_1$-adrenoceptors far less than to the $\beta$-adrenoceptors. Accordingly, this indicated that the $\alpha_1$-adrenoceptor population in rat heart is located more distantly from the nerve terminal than the $\beta$-adrenoceptor population.

Combined adrenoceptor stimulation was subjected to a cocaine-induced potentiation pattern similar to that for pure $\beta$-adrenoceptor stimulation. The potentiation of agonist expressed either as $T'_{max}$ or $T''_{min}/T'_{max}$ was approximately 10-fold. This reflects the dominating role of the $\beta$-adrenoceptors in mediating the combined response of rat papillary muscles to noradrenaline, as found in our earlier studies (Skomedal et al., 1988b).

In our experimental setting noradrenaline reached the receptors by passive diffusion from the medium in the organ bath. This might favour an influence of extraneuronal uptake mechanisms on the access of noradrenaline to the receptors. If such an effect was disproportional for different experimental groups, the results obtained could not be interpreted exclusively in terms of neuronal uptake blockade. To test this possibility we examined the effect of corticosterone on pure $\alpha$-, pure $\beta$- and combined adrenoceptor stimulation in the presence of cocaine. All three groups behaved similarily, exhibiting a small potentiation (2- to 5-fold) during extraneuronal uptake blockade. Thus there was no differential shift of the dose-response curves for the respective groups that could distort the interpretation of our main results.

Since the $\beta$-adrenoceptors in rat heart probably exhibit a larger spare receptor capacity than the $\alpha_1$-adrenoceptors, it must be considered whether this could contribute to the differential shifts of the concentration-response curves induced by cocaine. The relative change in the agonist concentration giving a certain inotropic response, corresponding to a certain amount of agonist-receptor complex, is, however, only dependent upon the relative change in availability of the agonist to the receptor, assuming that the number of receptors is constant. Thus the shift of the concentration-response curves induced by cocaine is not influenced by the spare receptor phenomenon.

The maximal responses, expressed as $T'_{max}$, were examined during pure $\alpha$-, pure $\beta$- and combined adrenoceptor stimulation. These results (Table 1) show that the maximal inotropic response in the rat heart during $\alpha_1$-adrenoceptor stimulation was about 50% of the maximal inotropic response during $\beta_1$-adrenoceptor stimulation.

There were no statistically significant changes in maximal inotropic responses in our experiments (Table 1) in the absence compared to the presence of either neuronal or both neuronal and extraneuronal uptake blockers. This shows that it is possible to achieve maximal values without uptake blockade and that the blockers themselves do not significantly influence the function of the papillary muscles. Thus, the method is suitable for studying the potentiating effects of uptake blockers.

Our results indicate that the $\alpha_1$-adrenoceptor population is located geometrically more distantly from the sympathetic nerve terminal than the $\beta_1$-adrenoceptor population in rat papillary muscles. Studies on papillary muscles in rabbit heart indicate a different pattern in rabbits (Skomedal et al., 1989). These findings may have functional implications. Rats are relatively dominated by the $\beta$-adrenoceptor system. The neurotransmitter noradrenaline will have more easier access to receptors located within the synaptic cleft than to receptors located more distantly from the nerve terminal, due to the very effective neuronal uptake of noradrenaline. Our results might indicate that neuronal and humoral adrenoceptor agonists may have differential effects on the $\alpha$-adrenoceptor system compared to the $\beta$-adrenoceptor system because of the relative location of these systems in rat hearts. The localisation of the $\beta$-adrenoceptor system strongly supports the prominent role of the $\beta_1$-adrenoceptor system in the rat myocardium. However, the $\alpha_1$-adrenoceptor population may play a minor role during weak sympathetic stimulation, but may be relatively more important during excessive nerve stimulation and during humoral stimulation by adrenaline.

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