NO EVIDENCE FOR INVOLVEMENT OF DOPAMINERGIC RECEPTORS IN THE POSITIVE INOTROPIC ACTION OF DOPAMINE ON THE ISOLATED RABBIT PAPILLARY MUSCLE

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Abstract—Experiments were carried out on the isolated rabbit papillary muscle driven at 0.5 Hz in order to further elucidate the mechanism of the positive inotropic effect evoked by dopamine. The dose-response curve for dopamine was not affected by the antagonists pimozide (10^{-6} M), yohimbine (10^{-5} M), pindolol (3 \times 10^{-8} M) and phentolamine (10^{-6} M), when these agents were given separately. Only the simultaneous administration of yohimbine plus pindolol and phentolamine plus pindolol, respectively, shifted the entire curve to the right. This shift was not further influenced by pimozide. Dopamine (10^{-4} M) increased the cyclic AMP content of the papillary muscle by about 50%; this increase was not affected by pimozide, but was markedly elevated by yohimbine and completely depressed by pindolol. From the present results it is concluded, that dopamine produces its positive inotropic effect through stimulation of myocardial \(\alpha\)- as well as \(\beta\)-adrenoceptors to about the same degree; stimulation of specific dopaminergic receptors, however, is not involved. The stimulation of \(\beta\)-adrenoceptors is accompanied by an increase of the cyclic AMP level, while that of \(\alpha\)-adrenoceptors is not.

Dopamine has been postulated to produce its positive inotropic effect by stimulation of cardiac \(\beta\)-adrenoceptors directly and/or indirectly via the release of noradrenaline from the nerve terminals (1). Recent studies from our laboratory, however, showed that in the isolated rabbit papillary muscle the positive inotropic effect evoked by dopamine was mediated not only by \(\beta\), but also by myocardial \(\alpha\)-adrenoceptor stimulation (2, 3). The entire dose-response curve for dopamine was neither affected by the \(\alpha\)-adrenolytic drug phentolamine nor by the \(\beta\)-adrenolytic drug pindolol, if given separately; the simultaneous administration of both \(\alpha\) and \(\beta\)-antagonists, however, led to a marked shift of the complete curve to the right. Thus, it has been assumed that dopamine stimulates myocardial \(\alpha\)- as well as \(\beta\)-adrenoceptors to about the same degree (3).

A substantial amount of evidence has accumulated that the positive inotropic effect caused by cardiac \(\beta\)-adrenoceptor stimulation is accompanied by an increase of the cyclic AMP content (4–7), while that via myocardial \(\alpha\)-adrenoceptors obviously is not (3, 6–10). Accordingly the dopamine induced increase of the cyclic AMP level of the isolated rabbit papillary muscle (3) and of the isolated perfused rat heart (11) is most likely due to its stimu-

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lation of β-adrenoceptors. However, in nervous tissues, elevated cyclic AMP levels were also found after stimulation of specific dopaminergic receptors (12–14). In order to determine whether this mechanism may also play a role in the positive inotropic action of dopamine, the influence of pimozide—a specific dopaminergic receptor antagonist (15, 16)—on the effects of dopamine on contractile force and on the cyclic AMP level of the isolated rabbit papillary muscle was investigated.

According to our recent observation (3) in the isolated rabbit papillary muscle, the increase in levels of the cyclic AMP induced by dopamine was markedly elevated by the α-adrenolytic drug phentolamine. Hence, the question arose whether these changes of the cyclic AMP increase are due to a specific action of dopamine in the presence of an α-adrenolytic drug or to a non-specific action of phentolamine, which is known to increase myocardial contractility through an effect on the sympathetic nervous system (17). Therefore, we also studied the influence of the α-adrenolytic drug yohimbine on the effects of dopamine on the cyclic AMP generating system.

MATERIALS AND METHODS

Papillary muscles of a diameter of 1 mm or less were excised from the right ventricle of rabbits of either sex weighing 1.8–2.5 kg. These tissues were mounted in a 20 ml organ bath containing Krebs-Henseleit solution with 0.057 mM ascorbic acid bubbled with 95% O₂ and 5% CO₂ at a temperature of 37°C. Such were electrically stimulated by square wave impulses at 5 msec duration and a voltage about 20% above threshold at a frequency of 0.5 Hz. The developed tension of the muscle under a resting tension of 0.5 g was recorded via a strain gauge on a Hellige recorder. In all experiments, papillary muscles were equilibrated for 60 min before any drug was administered.

After equilibration a submaximal effective concentration of dopamine (10⁻⁴ M) was administered twice or three times, until the successive response remained unchanged. Cumulative dose-response curves were determined by adding 0.1 ml of the drug solution thus increasing the final bath concentration in steps of 0.5 log units. When a steady state of the developed tension was reached—usually after 5 min—the next higher concentration was applied. After the maximal response was obtained, the muscles were washed every ten min for 60 min until the developed tension returned to the control value. In some experiments the antagonists pimozide (10⁻⁶ M or 10⁻⁵ M), phentolamine (10⁻⁸ M) or pindolol (3 × 10⁻⁸ M)—either separately or in combination—were present in the Krebs-Henseleit solution throughout the experiments; another series of experiments were performed in a Krebs-Henseleit solution containing yohimbine (10⁻⁵ M) and/or pindolol (3 × 10⁻⁸ M). Details of the experimental procedure have been described in a previous paper (18).

Cyclic AMP assay: 60 sec after the administration of a submaximal effective concentration of dopamine (10⁻⁴ M) the papillary muscles were removed from the organ bath, blotted with filter paper and frozen immediately in liquid nitrogen.

The muscles were weighed and homogenized in 0.3 ml of 5% trichloroacetic acid for 30 sec by use of a microdismembrator (B. Braun, Melsungen). The content of cAMP in
suitable aliquots was determined by the protein binding method of Gilman (19) as previously described (7). The recovery of a known amount (400 pmoles) of unlabelled cyclic AMP added to 1 ml of 5% trichloroacetic acid before the homogenization of muscles pretreated with the given drugs as well as untreated control muscles amounted to 97.4±8.9% (n=10); thus corrections were not necessary.

**Statistical methods:** The significance of differences was estimated by means of Student's t-test. P-values smaller than 0.05 are considered to be significant. The pD₂-values were calculated as described by Van Rossum (20).

**Drugs used:** Dopamine hydrochloride (Serva, Heidelberg); (-) Phenylephrine hydrochloride (Boehringer, Ingelheim); (+) Pindolol base (Sandoz, Basel); Phentolamine hydrochloride (CIBA, Basel); Pimozide (Janssen, Dusseldorf); Yohimbine hydrochloride (Boehringer, Ingelheim) and for assay: ³H-cyclic AMP (specific activity 38.4 Ci/mmol, New England Nuclear, Dreieichenhain).

Stock solution of dopamine was prepared in 1% ascorbic acid and kept ice-cooled in order to avoid autoxidation.

**RESULTS**

The contractile amplitude in the papillary muscle driven at 0.5 Hz was 170.9±11.1 mg (n=54). The maximum of the developed tension evoked by dopamine amounted to 781.3±23.4 mg (n=54). The dose-response curve for the positive inotropic effect of dopamine was not affected by the antagonists pimozide (10⁻⁶ M and 10⁻⁵ M), phentolamine (10⁻⁶ M) or pindolol (3×10⁻⁸ M), when these agents were given separately (Figs. 1 and 2). The same holds true for the combined application of pimozide (10⁻⁶ M) plus phentolamine (10⁻⁶ M, Fig. 2), pimozide (10⁻⁵ M) plus phentolamine (10⁻⁵ M, not shown) and pimozide (10⁻⁶ M) plus pindolol (3×10⁻⁸ M).

**FIG. 1.** Influence of 10⁻⁶ M (A) and 10⁻⁵ M (B) pimozide, respectively, on the positive inotropic effect of dopamine in the isolated rabbit papillary muscle in the presence and absence of pindolol (3×10⁻⁸ M). ○—○: control (n=54 in A and B). △—△: pindolol (n=11 in A and B). ●—●: pimozide (10⁻⁶ M) (n=8 in A). ■—■: pimozide (10⁻⁵ M) (n=11 in B). ▲—▲: pimozide (10⁻⁶ M) plus pindolol (n=5 in A). ▼—▼: pimozide (10⁻⁵ M) plus pindolol (n=15 in B).
plus pindolol (3 x 10^-8 M, Fig. 1A), respectively. In the presence of 3 x 10^-8 M pindolol a tenfold higher concentration of pimozide (10^-5 M), however, led to a shift of the whole curve to the right by about 0.4 log units (Fig. 1B). Thus, the pD2-value for dopamine in the presence of pimozide (10^-5 M) plus pindolol (3 x 10^-8 M, 4.43±0.05, n=15) was significantly lower than that for dopamine (4.84±0.02, n=54, p<0.001). As previously described, phentolamine (10^-6 M) plus pindolol shifted the dose-response curve for dopamine to the right by about 1.0 log unit (3, see also Fig. 3). This shift was not affected by pimozide (10^-6 M), but slightly increased by the ten fold higher concentration of pimozide (Fig. 3).

To determine whether the shift of the dose-response curve of dopamine induced by 10^-5 M pimozide in the presence of the ß-adrenolytic drug pindolol is due to an additional blockade of specific dopaminergic receptors or to a blockade of myocardial ß-adrenoceptors, the influence of pimozide on the dose-response curve for the positive inotropic effect of phenylephrine—a drug known to stimulate myocardial ß-adrenoceptors (18)—was investigated. The result is given in Fig. 4.

While 10^-6 M pimozide did not affect the dose-response curve of phenylephrine, the ten fold higher concentration led to a marked shift of the whole curve to the right. The pD2-value for phenylephrine in the presence of 10^-5 M pimozide (5.11±0.09, n=5) was significantly different from that for phenylephrine (5.89±0.03, n=14, P<0.001), indicating...
that higher concentrations of pimozide are able to block myocardial α-adrenoceptors.

In a crucial experiment we studied the effects of apomorphine—a drug known to stimulate specific dopaminergic receptors (1)—on the contractile force of the papillary muscle. Apomorphine used in concentrations from 10⁻⁶ M up to 10⁻⁴ M did not affect the tension developed of the papillary muscle. The developed tension amounted in the absence of apomorphine to 183.7 ± 16.9 mg (n=5) and in the presence of 10⁻⁴ M apomorphine to 190.7 ± 22.6 mg (n=5).

As described above for phentolamine (Fig. 2), yohimbine (10⁻⁵ M) also had no effect on the dose-response curve for the positive inotropic effect of dopamine (Fig. 5). In the presence of the β-adrenolytic drug pindolol (3x10⁻⁸ M), however, yohimbine shifted the entire curve to the right by about 0.7 log units. Under these conditions the pD₂-value (4.10 ± 0.06, n=9) was significantly different from that in the absence of the antagonists (4.84 ± 0.02, n=14, P<0.001). The maximum of the developed tension evoked by dopamine however, was not changed under all conditions studied.

In contrast to its effect on the dose-response curve for dopamine, yohimbine (10⁻⁵ M) led to a marked shift to the right of the dose-response curve for phenylephrine (not shown). The affinity for phenylephrine expressed by means of the pD₂-values was in the presence of yohimbine (4.70 ± 0.05, n=4) significantly lower than in the absence of the adrenolytic drug (5.89 ± 0.03, n=14, P<0.001). This result demonstrates that the concentration of yohimbine used in the present experiments is sufficient to block myocardial α-adrenoceptors.
In another series of experiments, we studied the influence of the antagonists pimozide, yohimbine and pindolol on the dopamine induced increase of the cyclic AMP content of the papillary muscle. For this purpose the muscles were exposed to dopamine (10^{-4} M) for 60 sec, since at this time, the rise of the cyclic AMP level was maximal (3). As seen in Table 1 increase of the cyclic AMP content evoked by dopamine (10^{-4} M) amounted to approx. 50%, and such is in accordance with previously reported data (3). This increase was not affected by pimozide (10^{-6} M), but was markedly elevated by yohimbine (10^{-5} M). On the contrary, pindolol (3 \times 10^{-8} M) completely depressed the increase of the cyclic AMP content produced by dopamine.

**DISCUSSION**

It has recently been demonstrated in the isolated rabbit papillary muscle that the positive inotropic effect evoked by dopamine is due to at least three components: the less important indirect stimulation of \( \beta \)-adrenoceptors via the release of noradrenaline from the nerve terminals and the main direct stimulation of \( \alpha \)- as well as \( \beta \)-adrenoceptors to about the same extent (2, 3). The present results confirm these observations. Neither the \( \alpha \)-adrenolytic drug yohimbine nor the \( \beta \)-adrenolytic drug pindolol affected the dose-response curve for the positive inotropic effect of dopamine, if given separately. The simultaneous administration of both \( \alpha \)- and \( \beta \)-adrenoceptor antagonists, on the contrary, led to a shift of the entire curve to the right by about 0.7 log units. These results support our previous view that dopamine has nearly the same affinity to myocardial \( \alpha \)- as well as \( \beta \)-adrenoceptors (3).

In addition to its ability to stimulate \( \alpha \)- and \( \beta \)-adrenoceptors dopamine has been reported
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to produce effects through stimulation of specific dopaminergic receptors. The existence of these receptors has been demonstrated in ganglia (12), brain (13), mesenteric, renal (21, 22) and coronary (23) arteries. Stimulation of these specific dopamine receptors, however, does not seem to be involved in the positive inotropic action of dopamine in the isolated rabbit papillary muscle. This conclusion appears to be justified since pimozide \(10^{-6}\) M—a specific dopamine receptor antagonist (15, 16)—did not influence the dose-response curve for the positive inotropic effect of dopamine, neither alone nor in the presence of phentolamine or pindolol (cf. Fig. 1A and 2). Also in the presence of both \(\alpha\) and \(\beta\)-adrenoceptor antagonists, which shifted the whole curve to the right by about 1.0 log unit, pimozide \(10^{-6}\) M had no effect (cf. Fig. 3). Moreover, the present experiments revealed that apomorphine, a well known dopaminergic receptor stimulating agent (1), used in concentrations up to \(10^{-4}\) M did not affect the tension developed. These observations are in favour of the idea, that in the rabbit papillary muscle, there exist no receptors specific to dopamine. Similar results have been obtained in isolated electrically driven guinea pig atria, where haloperidol another well known dopamine receptor antagonist did not affect the positive inotropic effect evoked by dopamine (24).

The shift of the dose-response curve of dopamine induced by \(10^{-5}\) M pimozide in the presence of the \(\beta\)-adrenolytic drug pindolol is most likely due to an \(\alpha\)-adrenolytic side effect of this relatively high concentration of pimozide. This assumption is supported by the fact that \(10^{-6}\) M pimozide did not influence the dose-response curve of phenylephrine, a drug known to stimulate myocardial \(\alpha\)-adrenoceptors (18), whereas \(10^{-5}\) M pimozide led to a marked shift of the entire curve to the right. Obviously pimozide acts as a specific antagonist to dopaminergic receptors in only a certain concentration range, whereas higher concentrations exert an additional \(\alpha\)-adrenolytic action.

A large body of evidence has accumulated that the positive inotropic effect through \(\beta\)-adrenoceptor stimulation is mediated by cyclic AMP (4–7, 25), while that via myocardial \(\alpha\)-adrenoceptors probably is not (3, 7–10). Accordingly, increase of the cyclic AMP content of the papillary muscle obtained 60 sec after the administration of dopamine \((10^{-4}\) M) is most likely due to its stimulation of \(\beta\)-adrenoceptors directly and/or indirectly by the release of endogenous catecholamines (cf. Table 1). This assumption is strongly supported by the fact that the \(\beta\)-adrenolytic drug pindolol completely prevented the increase of the cyclic AMP content induced by dopamine. In nervous tissues, increased cyclic AMP levels were also found after stimulation of specific dopaminergic receptors (12–14). In the present study, however, pimozide \((10^{-6}\) M) did not influence the increase of the cyclic AMP content evoked by dopamine. Therefore it is concluded that stimulation of receptors specific to dopamine is not involved in the production of elevated cyclic AMP levels in the isolated rabbit papillary muscle.

In the presence of the \(\alpha\)-adrenolytic drug yohimbine, the rise of the cyclic AMP content caused by dopamine was markedly enhanced. These results confirm recently reported data from our laboratory (3) where the same effect was obtained under the influence of another \(\alpha\)-adrenolytic drug, phentolamine. The present results also support our recently stated
assumption that in the particular case of dopamine, which has obviously the same affinity to myocardial $\alpha$- as well as $\beta$-adrenoceptors, after the blockade of one type of receptors, all dopamine molecules can act on the other type. Hence in the presence of an $\alpha$-adrenolytic drug dopamine behaves like a "pure" $\beta$-adrenoceptor stimulating agent; the increase of the cyclic AMP content, under these conditions about 100% (cf. Table 1), is comparable with that evoked by the pure $\beta$-adrenoceptor stimulating agent isoprenaline (3, 7).

The present results revealed that dopamine produces its positive inotropic effect on the isolated rabbit papillary muscle by stimulation of $\alpha$- as well as $\beta$-adrenoceptors to about the same degree; thus, dopamine has nearly the same affinity to both types of cardiac adrenoceptors. Stimulation of specific dopaminergic receptors, however, is not involved in the positive inotropic action of dopamine. The positive inotropic effect evoked by stimulation of $\beta$-adrenoceptors is accompanied by an increase of the cyclic AMP content, while that via $\alpha$-adrenoceptors is not.

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