THE DIRECT CARDIODEPRESSANT ACTION OF METHAMPHETAMINE IN ISOLATED CARDIAC MUSCLE

MASANOBU URABE, SETSUHARU FUJITA AND KOICHIRO TAKASAKI

Department of Pharmacology, Medical College of Miyazaki, Miyazaki, 889, Japan

(Received for publication December 20, 1975)

Effects of d-methamphetamine and tyramine were studied on several physiological parameters in isolated rat heart muscle preparations. The sinoatrial rate and electrically-induced ventricular contractile force were suppressed dose-dependently by methamphetamine, although both the activities of cardiac muscle were effectively enhanced by tyramine. Clear-cut differences between the two amines were also demonstrated in their effect on ventricular excitability and functional refractoriness. Methamphetamine caused depressed excitability and increased refractoriness of cardiac muscle, whereas tyramine produced no change in its excitability, rather causing decreased refractoriness. It could be suggested from these results that methamphetamine is a direct cardiodepressant rather than a cardiac stimulant. In view of the structure-activity relationship, the direct cardiodepressant effect of methamphetamine was presumed to be basically related to so-called direct membrane effect.

There are a number of pharmacological studies on the peripheral effects of amphetamine and the analogues, but still controversy exists concerning the cardiovascular action. It is generally believed that these amines can displace norepinephrine from the sympathetic nerve terminals and thereby cause a large increase in systemic arterial pressure\(^1\). The recent papers, however, indicated that repeated doses of methamphetamine or ephedrine at sufficiently short intervals induced not only the diminution of pressor response, i. e., tachyphylaxis but hypotensive response in the intact animal\(^2\).\(^3\)\(^4\). Although tachyphylaxis may be explained by a decrease in the amount of norepinephrine released from the sympathetic nerve terminals induced by the amines, the hypotensive effect is not necessarily explainable by such a change in adrenergic function\(^5\).

On the other hand, considering the current concept that these amines are much less potent than norepinephrine in the vasoconstrictive activity, it seems likely that the cardiac effect may be possibly predominant in producing the changes of arterial pressure. Curiously enough, however, most of the work dealing with the drugs has not focussed on physiological properties of the amines for the isolated heart muscle preparation.

Accordingly, in this series of experiments, the effects of d-methamphetamine on several physiological parameters
are investigated in isolated rat heart muscle and compared to those of tyramine, an indirect adrenergic activator.

The direct cardiodepressant effect of methamphetamine is disclosed and its possible relationship to the cardiovascular action is discussed in the present paper.

METHODS

Male Wistar rats (about 250 g) were sacrificed without anesthesia. The hearts were quickly excised so as not to impair the region of sinus and atria. Then, the sinoatrial portion was isolated in oxygenated Krebs-Ringer solution warmed up to 37°C. A preload of 0.5 g was put on these preparations and contractile force was recorded with an isometric strain gauge (Nihon Kohden) connected to a pen-recorder. Most of the preparations showed spontaneous rhythmic contractions at a rate of 249 beats per min in average after the equilibration of 30 minutes.

On the other hand, narrow ventricular strips were prepared by dissecting left ventricular wall. The preparation was also placed in oxygenated medium kept at 37°C. These preparations, quiescent after a thirty minutes-period of equilibration, were electrically stimulated through platinum wire electrodes with cathodal square wave of 3 msec duration and five times the threshold strength at a rate of 0.5 Hz. The contraction tension under 1 g load was similarly recorded with a strain gauge. Estimation of ventricular functional refractory period was carried out as described by Govier. The drugs used were dissolved in Krebs-Ringer solution, neutralized by HCl. Krebs-Ringer solution employed in this series of experiments had the following composition (mM): NaCl 125, KCl 5, CaCl₂ 2.6, MgSO₄ 1.3, NaH₂PO₄ 1.25, NaHCO₃ 24, Glucose 10. The bathing solution was bubbled by a mixture of 95% O₂ and 5% CO₂, and the pH of medium was practically 7.4.

RESULTS

*Effect of methamphetamine and tyramine on sinoatrial rate of contraction*

Fig. 1 Effect of methamphetamine and tyramine on the sinoatrial rate of contraction. Arrows indicate the time when drugs were added. Methamphetamine (upper) and tyramine (lower) are indicated by abbreviated expression as MA and TY in all the figures of this communication.
The rhythmic contraction of sinoatrial preparations remained practically unchanged after equilibration. Addition of methamphetamine \((5 \times 10^{-4} \text{M})\) exhibited only minor facilitation, rather followed by a distinct decrease in the spontaneous rate 5 min later as shown in Fig. 1. By contrast, tyramine \((5 \times 10^{-4} \text{M})\) produced a considerable increase in the rate of spontaneous beats immediately after addition of the drug (Fig. 1).

In similar experiments, practolol \((1.5 \times 10^{-4} \text{M})\) was added 5 min prior to either methamphetamine or tyramine exposure in order to differentiate the intrinsic effect from the indirect effect, i.e., a sympathomimetic action on isolated sinoatrial pacemaker. Practolol by itself caused no change in the spontaneous rate, but this agent antagonized against the stimulating action of methamphetamine and tyramine. These experiments are graphically illustrated in Fig. 2, indicating that the positive chronotropic effect of the amines was obviously inhibited in the presence of practolol though the negative chronotropic effect observed after 10 min exposure to methamphetamine was essentially unaffected. Worthy of note is that methamphetamine elicits only inhibition of the spontaneous rate by the presence of practolol, implying the intrinsic property of methamphetamine to induce cardiac slowing. In Fig. 3, the dose-dependent

![Fig. 2 Changes in spontaneous rate of sinoatria induced by drugs.](image)

The effects of tyramine \((5 \times 10^{-4} \text{M})\) and methamphetamine \((5 \times 10^{-4} \text{M})\) on sinoatrial rate were observed under the presence or absence of practolol \((1.5 \times 10^{-4} \text{M})\). Experiments were performed as shown in Fig. 1. Open squares or circles indicate the effect of tyramine or methamphetamine alone, respectively. Closed squares and circles show the two drug effects modified by the presence of practolol. Each value is shown by the average of 4 experiments.

![Fig. 3 Effect of increasing concentrations of methamphetamine and tyramine on sinoatrial rate.](image)

The concentrations of methamphetamine (open circles) and tyramine (open squares) were increased from \(5 \times 10^{-4} \text{M}\) to \(2 \times 10^{-3} \text{M}\). Per cent change in the sinoatrial rate of contraction was measured after 5 minutes exposure to the respective drug. Each value is indicated by the average of 6 experiments.
Fig. 4 Effect of methamphetamine and tyramine on electrically-induced contraction tension of ventricular muscle.

Ventricular muscle strip was electrically stimulated as described in Methods. Ventricular contractile response was observed for 20 minutes after addition of methamphetamine ($5 \times 10^{-4}$ M, upper) or tyramine ($5 \times 10^{-4}$ M, lower).

Fig. 5 Effect of increasing concentrations of methamphetamine and tyramine on ventricular contractility.

Experiments were carried out similarly to Fig. 4, but the drug concentration was stepwisely increased every 5 min as indicated by arrows in the figure. The upper illustration represents the effect of increasing methamphetamine concentration on ventricular contractility. The lower is a typical case of tyramine.
effects of the drugs on sinoatrial rate are shown. Methamphetamine inhibited the spontaneous activity of sinoatria in a dose-dependent manner, whereas tyramine enhanced the spontaneous activity with the exception of its effect at higher concentrations.

**Effect of methamphetamine and tyramine on ventricular contractility and electrical threshold**

Ventricular strips were electrically stimulated with five times the threshold strength and then exposed to the amines. Addition of methamphetamine ($5 \times 10^{-4}$ M) produced no appreciable change in the contractile response of ventricular muscle even after 5 min exposure. While tyramine ($5 \times 10^{-4}$M) produced a significant increase in ventricular contractile force, occasionally associated with development of automatic contractions. Fig. 4 represents typical cases of these experiments.

Then, the respective concentration of two amines was increased stepwisely every 5 min and the cumulating effect on ventricular contractility was observed as indicated in Fig. 5. Ventricular contraction tension was progressively reduced while methamphetamine concentration was raised cumulatively. On the contrary, ventricular contractile force was dose-dependently increased by tyramine.

In the following experiments, the effect of these amines on ventricular excitability was studied by measuring electrical threshold and functional refractoriness, since the reduced contractile response in the presence of methamphetamine was significantly restored by increasing the intensity of electrical stimuli. The minimal voltage required to provoke full contraction tension was determined, and excitability of ventricular muscle was expressed by the reciprocal of threshold voltage. As seen in Fig. 6, methamphetamine caused a dose-dependent reduction in the excitability of ventricular muscle, but tyramine did little or not.

On the other hand, paired-pulse stimulation was applied to ventricular muscle at a rate of 0.3 Hz, and the interval of a pair of pulses was gradually shortened until an amplitude of the second contraction reduced by a half size of the first, also until the second response failed to appear. Thus, the relative and absolute functional refractory periods were estimated to be 190.6 ± 8.16 and 72.9 ± 3.26 (S.E.) msec, respectively. The drug effects observed after 20 min exposure are listed in Table 1, showing that methamphetamine...
TABLE 1
Effect of methamphetamine and tyramine on functional refractory period
in ventricular muscle

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Concentration</th>
<th>Relative F.R.P.</th>
<th>Absolute F.R.P.</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>190.6 ± 8.16 (18)</td>
<td>72.9 ± 3.26 (16)</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>5 × 10⁻⁴M</td>
<td>253.0 ± 10.90 (5)</td>
<td>82.8 ± 4.00 (6)</td>
</tr>
<tr>
<td></td>
<td>10⁻³M</td>
<td>338.0 ± 23.74 (5)</td>
<td>102.0 ± 2.00 (3)</td>
</tr>
<tr>
<td>Tyramine</td>
<td>5 × 10⁻⁴M</td>
<td>116.0 ± 3.78 (5)</td>
<td>63.8 ± 5.15 (4)</td>
</tr>
<tr>
<td></td>
<td>10⁻³M</td>
<td>120.0 ± 2.90 (3)</td>
<td>72.7 ± 1.45 (3)</td>
</tr>
</tbody>
</table>

Paired-pulse stimulation was employed for estimation of functional refractoriness. Number of preparations is indicated in brackets.

significantly increased ventricular refractoriness but tyramine decreased it inversely.

DISCUSSION

As shown in the present study, tyramine evoked not only clear-cut enhancement in the pacemaker activity of isolated sinicatria but also significant increase in the contractile force of ventricular muscle, suggesting that the cardiac muscle preparation placed in oxygenated Krebs-Ringer solution kept at 37°C can serves as a suitable model for the research on indirectly acting amines. The observed positive chronotropic and inotropic effect induced by tyramine should be attributed entirely to the norepinephrine released from sympathetic nerve terminals involved in the preparation, since tyramine has been identified to be a typical in directly acting amine which owes its effect to the liberation of adrenergic transmitters.

On the other hand, methamphetamine is also classified as a tyramine-like drug, although the distinction between directly acting and indirectly acting amines is not absolute. Unexpectedly, however, methamphetamine caused only minor facilitation in the sinoatrial spontaneous activity followed by its depression in the later stage. Pretreatment with practolol revealed more consistently the suppressing effect of this amine, impling that methamphetamine exerts its inhibitory action by decreasing the slope of diastolic depolarization in the sinoatrial pacemaker cells. It was, furthermore, recognized in this study that methamphetamine produced no significant increase in the contraction tension of ventricular muscle but, contrary to tyramine, caused its dose-dependent depression. However, the contraction tension could be recovered to a good extent from the inhibitory action of methamphetamine if the strength of electrical stimulus was raised sufficiently. It is, therefore, unlikely that the reduced contractility by methamphetamine could be ascribed to any dysfunction of the contractile system of myocardium.

The present experiments focussed attention further on the comparison of methamphetamine and tyramine in the physiological action on ventricular excitability and refractoriness. Dissimilarities between methamphetamine and tyramine were pointed out in the effects on both parameters as follows. The former drug produced the dose-dependent decrease in ventricular excitability,
but the latter did not practically. Also, the two drugs were quite different from each other in their effect on refractoriness. Methamphetamine prolonged the functional refractory period, but tyramine shortened it inversely. Thus, the comparative study per se brings out clearly the intrinsic profile of methamphetamine with respect to its physiological property on excitable tissues. Namely, these results indicate a clear-cut disparity between the two amines: methamphetamine lowers directly the electrical excitability of cardiac muscle, whereas tyramine dose not possesses such a direct action, having only sympathomimetic action. It is evidently that methamphetamine is much less potent in sympathomimetic efficacy at least on the heart but has a direct cardiodepressant action. This finding would suggest that its cardiac effect is a minor factor in producing hypertensive response but rather contributable in significant hypotension caused by repeated doses of the drug, even though central or vascular contribution to its hypotensive effect is not negligible. On the other hand, it is of interest to speculate that the cardiodepressant action of methamphetamine may be basically related to so-called direct membrane action, viz., a membrane stabilizing action. If the speculation is true, the finding of this intrinsic property may give a hot lead in elucidating such basic mechanisms as blockade of catecholamine-uptake. In general, membrane stabilizers, e.g., phenothiazine derivatives are known to share the direct depressant effect on myocardium and also the inhibitory effect on mechanisms of catecholamine-uptake. Consequently, it seems presumable that both the cardiodepressant effect and the blockade of catecholamine-uptake by methamphetamine might be causally linked to its 'direct membrane effect'. Considering the structure-activity relationship, methamphetamine must be a high lipophilic compound to have a potent membrane effect since a close correlation between the direct membrane effect and the lipophilic character has been pointed out in the above-mentioned drugs. Methamphetamine has an unsubstituted phenyl ring in the molecular structure, which may characterize its hydrophobicity as a membrane stabilizer. Likewise, tyramine is also a phenylethylamine derivative similar to methamphetamine, but this amine has a hydroxyphenyl ring instead of unsubstituted one. Since para-hydroxylation of aromatic ring leads to losing its lipophilic property, then it seems assumable that the inability of tyramine as a direct cardiodepressant could be referred to this difference in its aromatic portion. In this connection, of further interest is that hydroxymphetamine, differing from amphetamine, has a potent cardiac stimulating effect, because tyramine is lacking of the depressant action but is a potent cardiac stimulant. Therefore, these findings would imply that the individual diversity in the amine effects could be at least in part traced back to the chemical structure of the aromatic portion.

In conclusion, it should be pointed out that methamphetamine can produce depressed excitability and increased refactoriness of myocardium, which might be responsible for its negative chronotropic and inotropic effect. This direct cardiodepressant effect of methamphetamine might be basically linked to a 'direct membrane effect'.

REFERENCES