The Effects of Cocaine and Metanephrine on the Cardiac Responses to Sympathetic Nerve Stimulation in Dogs

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With the assistance of Herrick Finkelstein

SUMMARY The effects of infusions of cocaine (COC) and metanephrine (MET) on the inotropic and chronotropic responses to cardiac sympathetic nerve stimulation were studied in open-chest, anesthetized dogs. COC blocks the neuronal uptake of norepinephrine (NE), whereas MET blocks the extraneuronal uptake. Both blocking agents slightly enhanced the inotropic but not the chronotropic responses. COC prolonged the cardiac responses significantly, particularly the chronotropic responses, whereas MET had no appreciable effect on the durations of these responses. Hence, it appears that the neuronal uptake mechanism plays a major role in the dissipation of neurally released NE in the heart, but that the extra-neuronal uptake mechanism plays only a minor role in its dissipation. In contrast to the results in certain other tissues, the combined effects of COC and MET on the cardiac responses were no greater or more prolonged than the sum of the effects produced by each agent acting alone.

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THE norepinephrine (NE) released at postganglionic sympathetic nerve terminals is dissipated by two principal mechanisms: (1) tissue uptake, and (2) diffusion into the blood stream (Axelrod and Weinshilboum, 1972). In the heart, tissue uptake appears to be the principal mechanism for terminating the effects of sympathetic neural activity (Stjärne and Wennmalm, 1971). The released NE is taken up both by the nerve endings themselves and by extraneuronal tissues.

Several chemicals are potent inhibitors of the neuronal uptake of NE. Cocaine (COC) is the prototype of such agents, and it has been used widely to assess the role of neuronal uptake in the sympathetic neural regulation of cardiac function (Johnson and Kahn, 1966; Koerker and Moran, 1971; Gillespie, 1973; Iversen, 1975). In previous studies by Johnson and Kahn (1966), by Koerker and Moran (1971), and from our laboratory (Levy and Blattberg, 1978), COC did not augment significantly, but did prolong, the inotropic and chronotropic responses to cardiac sympathetic nerve stimulation.

Metanephrine (MET) is one of the potent inhibitors of the extraneuronal uptake of NE (Iversen, 1965, 1975; Iversen et al., 1966; Gillespie, 1973). Bacq and Renson (1961) reported that MET potentiated the contraction of the nictitating membrane of the cat in response to sympathetic nerve stimulation. Langer et al. (1967) also found that the response of the nictitating membrane to the combination of sympathetic stimulation and MET was greater than that to neural stimulation alone. The combined effects of sympathetic stimulation and MET were essentially additive in their experiments. The effects of other extraneuronal blocking agents, such as adrenocortical steroids, have been studied in a variety of tissues, but the results have been conflicting. A pronounced interaction between the neuronal and extraneuronal uptake mechanisms was obtained in experiments on certain tissues (Kalsner, 1969; Hughes, 1972; Kaumann, 1972), such that the effects of combined blockade were much greater than the sum of the effects produced by separate blockade of the two uptake mechanisms. However, the interaction was insignificant in other structures (Margolius et al., 1967; Junstad et al., 1973).

The present experiments were conducted in intact, open-chest dogs to study the responses of the heart to certain agents that block the neuronal and extraneuronal uptake mechanisms. We have determined also the effects of combined blockade on such cardiac responses.

Methods

Experiments were conducted on 25 mongrel dogs that were anesthetized with sodium pentobarbital, 30 mg/kg, iv. A tracheal cannula was inserted through a midline cervical incision, and intermittent positive pressure ventilation was begun. Both
vagi were crushed by tight ligatures at the mid-cervical level. The chest was opened bilaterally through a transverse incision in the 4th intercostal space. The upper poles of both stellate ganglia were crushed by tight ligatures to interrupt almost all of the tonic sympathetic neural activity to the heart (Levy et al., 1966). Shielded iridium electrodes (Harvard Apparatus Co.) were applied to both limbs of the right ansa subclavia.

Arterial blood pressure was measured from a femoral artery by means of a Statham transducer (P23AA). A Walton-Brodie strain gauge arch was used to measure the myocardial contractile force. It was attached to the wall of the right ventricle, parallel, and about 1 cm lateral, to the anterior descending coronary artery at a site about halfway between the apex and base of the heart. Cardiac cycle length was derived electronically from the strain gauge arch output. Arterial blood pressure, right ventricular contractile force, and cardiac cycle length were recorded on a direct-writing Brush oscillograph (Mark 260).

Each dog was assigned to one of the groups listed in Table 1. A randomization scheme was used such that there would be an equal number (n = 5) of dogs in each group. In each of three experimental periods, regardless of the experimental group, stimuli were delivered to the decentralized right ansa subclavia at frequencies of 0.5, 2, and 8 Hz (Grass stimulator, model S9). Stimulation at each frequency consisted of a 1-minute train of rectangular pulses, 2 msec in duration, and of supramaximal voltage (usually 15 V, monitored on an oscilloscope). The order of applying these frequencies was randomized in each experimental period.

During the first experimental period in all dogs, the changes in contractile force, cardiac cycle length, and arterial blood pressure in response to the various frequencies of stimulation were determined in the absence of any uptake-blocking drugs. During the second experimental period, group U (the untreated control group) received no blocking drugs. In groups CC and CM, cocaine hydrochloride was infused intravenously at a rate of 330 μg/kg per min for a 15-minute period and then at a rate of 66 μg/kg per min for the remainder of the experiment. In groups MM and MC, MET was infused intravenously at a rate of 50 μg/kg per min for a 10-minute period and then at a rate of 20 μg/kg per min for the remainder of the experiment. The quantity of MET infused per unit body weight during the initial 10-minute period was equal to that injected by Bacz and Renson (1961) in their studies on anesthetized cats. Twenty minutes after the beginning of the COC or MET infusion, the cardiac responses to ansal stimulation were determined at each stimulation frequency.

During the third experimental period, the infusion of COC was continued at a rate of 66 μg/kg per min in groups CC and CM. In group CM, MET was infused in addition to COC; the regimen for administering the MET was identical to that used in groups MM and MC during the second experimental period. In groups MM and MC, the infusion of MET was continued at a rate of 20 μg/kg per min throughout the third experimental period. In group MC, COC was infused in addition to MET; the regimen for administering COC was identical to that used in groups CC and CM during the second experimental period. Twenty minutes after the beginning of the third experimental period, the cardiac responses to ansal stimulation again were determined.

The data were analyzed by means of a mixed model analysis of variance (Sokal and Rohlf, 1969). The fixed effect factors were the experimental groups, experimental periods, and stimulation frequencies, whereas the individual dogs constituted a random effect factor.

**Results**

**Responses to Sympathetic Stimulation in a Representative Experiment.**

Figure 1 shows the changes in right ventricular contractile force, cardiac cycle length, and arterial blood pressure elicited by stimulation of the right ansa subclavia in a representative experiment. The record was obtained during the first experimental period in the absence of COC or MET. Ansal stimulation (between the arrows) evoked a 157% increase in contractile force, a 162-msec reduction in cardiac cycle length, and a triphasic change in arterial blood pressure. After cessation of stimulation, these variables returned toward their control levels. The half-times for recovery were 29 seconds and 36 seconds for contractile force and cycle length, respectively. These values are the times required for the responses to return halfway to their steady state recovery levels, and they reflect the durations of the responses after cessation of stimulation. The composite data for the responses to ansal stimulation during the various experimental periods will be described after the next section.
Effects of COC and MET Infusions

The changes in ventricular contractile force, cardiac cycle length, and arterial blood pressure in response to the infusions of COC and MET are shown in Figure 2. The data were analyzed by a mixed model analysis of variance (Table 2). Experimental groups and periods were considered to be fixed effect factors, and the individual dogs were considered to be a random effect factor. The cardiovascular variables were measured at the end of period 1 (P₁) for each group and at the end of the initial 15-minute infusion of COC during period 2 (P₂) for groups CC and CM, at the end of the initial 10-minute infusion of MET for groups MM and MC, and at the end of the first 15-minute period of observation during P₂ for the control group (U).

In each dog, contractile force at the end of P₁ was arbitrarily taken as 100% for the purposes of this analysis (Fig. 2, left panel). Contractile force was significantly greater at P₂ than at P₁ (Table 2, P = 0.05), but the differences among the various groups were not significant. Similarly, cardiac cycle length (Fig. 2, middle panel) was significantly greater at P₂ than at P₁ (P = 0.05), but again the differences among the various groups were not significant (Table 2).

In control group U, the arterial blood pressure (Fig. 2, right panel) was about 105 mm Hg and did not change appreciably from P₁ to P₂. The infusions of COC in groups CC and CM evoked blood pressure elevations of 14 and 22 mm Hg, respectively, whereas the infusions of MET in groups MM and MC produced rises of 46 and 38 mm Hg, respectively (Fig. 2). The differences between periods and among groups (Table 2) were highly significant (P < 0.001 and P = 0.01, respectively), and the interaction between these factors also was highly significant (P = 0.001). The blood pressure changes in the groups that received COC and in those that received MET were significantly different from the changes in group U (P = 0.01 and P = 0.001, respectively) on the basis of a "planned comparison" test (Sokal and Rohlf, 1969, p. 226).

Responses to Sympathetic Nerve Stimulation; Composite Data

The cardiac responses to ansa stimulation during the various experimental periods are shown in Figures 3–7, and the analysis of variance is presented in Table 3.

Magnitude of the Inotropic Responses

In all groups, the magnitude of the contractile force response increased with the frequency of neural stimulation (Fig. 3, left panel). The mean squares among groups (G), among periods (P), and among frequencies (F) were all highly significant (Table 3). There was a significant interaction (P = 0.01) between F and G; i.e., there was a significantly different response to sympathetic neural stimu-
TABLE 2  Analysis of Variance of the Changes in Contractile Force, Cardiac Cycle Length, and Arterial Blood Pressure Evoked by Infusion of Cocaine (5 mg/kg) or Metanephrine (0.5 mg/kg)

<table>
<thead>
<tr>
<th>Source</th>
<th>Groups</th>
<th>Degrees of freedom</th>
<th>Contractile force</th>
<th>Mean square</th>
<th>F ratio</th>
<th>Cycle length</th>
<th>Mean square</th>
<th>F ratio</th>
<th>Arterial blood pressure</th>
<th>Mean square</th>
<th>F ratio</th>
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<td></td>
<td></td>
<td>4,16</td>
<td>220</td>
<td>0.66</td>
<td></td>
<td></td>
<td>2,906</td>
<td>0.46</td>
<td></td>
<td>1,464</td>
<td>5.25*</td>
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<tr>
<td>Periods</td>
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<td>3,966</td>
<td>13.25†</td>
<td></td>
<td></td>
<td></td>
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<td>11.16†</td>
<td></td>
<td>7,838</td>
<td>142.15‡</td>
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<tr>
<td>Interaction</td>
<td>4,16</td>
<td>220</td>
<td>0.66</td>
<td></td>
<td></td>
<td></td>
<td>231</td>
<td>0.99</td>
<td></td>
<td>751</td>
<td>10.71‡</td>
</tr>
</tbody>
</table>

† P = 0.05; * P = 0.01; † P < 0.001.

Duration of the Inotropic Responses

The durations of the contractile force responses, as assessed by the 50% recovery time, increased with the frequency of anodal stimulation (Fig. 4, left panel), and they varied from period to period (middle panel). The mean squares among groups, periods, and frequencies were all highly significant (P < 0.01, Table 3). Also, there was a highly significant interaction (P < 0.001) between groups and periods (Table 3); i.e., the responses to sympathetic stimulation during the three experimental periods were significantly different among the various groups of dogs. When COC was given initially (groups CC and CM) during P2, there was a marked prolongation of the inotropic response (Fig. 4, middle panel). When COC was not given until P3 (group MC), the prolongation did not occur until P3. The infusion of MET had only a negligible effect on the duration of the inotropic response (P2 and P3, group MM; P2, group MC). When MET was infused along with COC (P3, group CM), the prolongation was augmented.

Magnitude of the Chronotropic Responses

The magnitude of the positive chronotropic response to cardiac sympathetic stimulation varied with the frequency of stimulation (Fig. 5, left panel); the mean square for stimulation frequency was highly significant (P < 0.001). The mean squares among groups and among periods were not significant (Table 3). This signifies that, in general, the magnitude of the chronotropic response to anodal stimulation was not significantly influenced by...
either COC or MET. There was a significant interaction ($P = 0.01$) between periods and groups, principally reflecting the attenuation of the chronotropic response in $P_1$ (group MC) when COC was infused along with MET (Fig. 5, middle panel). The physiological meaning of this observation is not apparent, however, because a similar attenuation was not observed when the infusion of MET was combined with that of COC ($P_3$, group CM).

**Duration of the Chronotropic Responses**

The durations of the cycle length responses, as assessed by the 50% recovery time, varied directly with the sympathetic stimulation frequency (Fig. 6, left panel). The changes varied considerably among the different groups and periods; the mean squares among groups, periods, and frequencies were all highly significant ($P < 0.001$, Table 3). All the first-order interactions were also highly significant ($P < 0.001$); i.e., the responses to the three stimulation frequencies differed among the various groups (Fig. 6, left panel) and among the different periods (right panel), and the responses during the three experimental periods differed among the various groups (center panel).

The principal reason for these results is that COC had a dramatic effect on the duration of the chronotropic response to ansal stimulation. When COC was infused initially during $P_2$ (Fig. 6, center panel, groups CC and CM), the major prolongation occurred during $P_2$, whereas when COC was not infused until $P_3$ (group MC), the significant prolongation did not occur until that period. Infusion of MET alone ($P_2$ and $P_3$ in group MM; $P_2$ in group MC) had no significant effect on the duration of the chronotropic response.

It may be noted that the second-order interaction ($G \times P \times F$) is also highly significant ($P < 0.001$) for the duration of the chronotropic response (Table 3). The second-order interaction may be interpreted in several ways, but one such interpretation is that the extent of the interaction between groups and

![Figure 4](http://circres.ahajournals.org/)

**Figure 4** The effects of COC and MET infusions on the durations of the right ventricular contractile force responses to cardiac sympathetic stimulation. The durations are measured as the 50% recovery time, i.e., the time required for the inotropic response to return halfway to its steady state recovery level after cessation of neural stimulation. The panels represent the $F \times G$, $G \times P$, and $F \times P$ interactions, as described in Figure 3. Statistical analysis in Table 3.

![Figure 5](http://circres.ahajournals.org/)

**Figure 5** The effects of COC and MET infusions on the magnitudes of the cardiac cycle length responses to cardiac sympathetic stimulation. These chronotropic responses are expressed as the change in cycle length from the prestimulation control value. The panels represent the $F \times G$, $G \times P$, and $F \times P$ interactions, as described in Figure 3. Statistical analysis in Table 3.
The NE liberated at the sympathetic nerve endings in the heart is removed by neuronal or extra-neuronal uptake or by diffusion into the myocardial capillary bed. In the present study, infusion of COC, a potent neuronal uptake blocking agent, caused some elevation of blood pressure (Fig. 2) and a slight augmentation of the inotropic response to anodal stimulation (Fig. 3) but no appreciable effect on the magnitude of the chronotropic response to anodal stimulation (Fig. 5). Its principal effect was to prolong the inotropic (Fig. 4) and especially the chronotropic responses (Figs. 6 and 7) to anodal stimulation. The infusion of MET, a potent extra-neuronal uptake-blocking agent, caused a more pronounced elevation of blood pressure than did COC at the infusion rates that were employed (Fig. 2). MET slightly augmented the inotropic response to anodal stimulation (Fig. 3), but had no detectable effects on the duration of the inotropic response (Fig. 4) or on the magnitude or duration of the chronotropic response (Figs. 5 and 6).

On the basis of the relative changes in the durations of the inotropic and chronotropic responses evoked by COC and MET, it may be concluded that, in the heart, the neuronal uptake processes are more important than are the extra-neuronal uptake processes in the removal of neurally released NE. It may be presumed that, after the neuronal uptake process has been blocked by COC, the neurotransmitter remains longer in the synaptic clefts, thereby prolonging the cardiac responses. Such prolongations have been reported previously (Johnson and Kahn, 1966; Koerker and Moran, 1971; Levy and Blattberg, 1978).

Suppression of neuronal reuptake would also tend to increase the concentration of neurotransmitter in the synaptic clefts, because the concentration depends on a dynamic balance between the rates of release and removal. Such an increase in NE concentration would tend to augment the magnitude of the cardiac responses. The magnitude of the chronotropic response was not affected significantly by COC (Fig. 5), however, and the enhancement of the inotropic response was not pronounced (Fig. 3). In other studies, it has been observed that COC does tend to enhance the cardiac responses to sympathetic neural stimulation in vitro preparations (Hukovic and Muscholl, 1962; Furchgott et al., 1963; Gillis and Schneider, 1967; Starke and Schumann, 1972; Chang and Lee, 1973; McCulloch et al., 1974). In one study on the intact dog (Moore, 1966), it also was found that COC enhanced the cardiac responses to sympathetic stimulation, but in four other studies in intact animals (Johnson and Kahn, 1966; Koerker and Moran, 1971; Levy and Blattberg, 1976, 1978), the responses were not augmented appreciably. At the dosage levels of COC usually used in vivo, it is likely that the tendency to increase NE concentrations at the receptor sites produced by suppression of neuronal reuptake is counteracted by the tendency for COC also to depress the neurotransmitter release process, perhaps by virtue of its local anesthetic property (Koerker and Moran, 1971; Hughes, 1972; Iversen, 1975).

MET is one of the most potent blocking agents of the extra-neuronal uptake process for NE (Iver-
It does possess other cardiovascular actions as well. The methoxy metabolites of epinephrine and norepinephrine, namely MET and normetanephrine (NMN), have direct agonistic activity on both \( \alpha \)- (Khairallah et al., 1966; Langer and Rubio, 1973) and \( \beta \)- (Langer and Rubio, 1973) receptors. They also may alter the responsiveness of the \( \beta \)-receptors to NE and epinephrine (Dresse and Lecomte, 1960; Chrusciel et al., 1965). The methoxy metabolites also have an indirect action, in that they release neurotransmitter from the peripheral sympathetic nerve endings (Khairallah et al., 1966; Langer and Rubio, 1973).

It is likely that, at the rates of administration used in our experiments, the tissue concentrations of MET were adequate to achieve a pronounced suppression of the extraneuronal uptake of NE. The infusion of MET during \( P_2 \) evoked a 40 mm Hg increase in the arterial blood pressure but only negligible effects on cardiac contractile force and cycle length (Fig. 2; Table 2). In the absence of appreciable cardiac effects, therefore, the elevation of blood pressure probably was produced principally by an increase in peripheral vascular resistance. The studies of Khairallah et al. (1966) indicate that the threshold concentration of MET necessary to constrict vascular smooth muscle is about \( 5 \times 10^{-5} \) M. Hence, the substantial arterial blood pressure elevation in our experiments evoked by the infusion of MET probably denotes that the tissue concentrations were in excess of this threshold value. Much lower concentrations have been found to inhibit the extraneuronal uptake mechanism for NE. In experiments on isolated rat heart, for example, a concentration of \( 2.9 \times 10^{-5} \) M produced a 50% inhibition of the extraneuronal uptake of NE (Burgen and Iversen, 1965; Iversen, 1975).

At the infusion rates used in our experiments, MET had no significant influence on cardiac contractile force or cycle length (Fig. 2, Table 2). In isolated heart preparations, it has been shown that MET and NMN do have inotropic and chronotropic effects, but it is necessary to use concentrations about 1000 times greater than the effective concentration for NE (Holtz et al., 1960; Chrusciel et al., 1965; Langer and Rubio, 1973). Such cardiac responses to high concentrations of the methoxy metabolites are probably mediated through the \( \beta \)-receptors, because they can be blocked with propranolol (Langer and Rubio, 1973).

In our experiments, the inotropic responses to sympathetic stimulation (Fig. 3, middle panel) were significantly greater after MET (\( P_2 \)) than before MET (\( P_1 \)), but the chronotropic responses were not significantly affected (Fig. 5). The mechanism responsible for these enhanced inotropic responses has not been determined. Junstad et al. (1973) observed that NMN increased the overflow of NE from the perfused rabbit heart by about 25% in response to sympathetic nerve stimulation. This suggests that inhibition of extraneuronal uptake with a consequent increase in neurotransmitter concentration in the synaptic cleft may be involved in the enhancement of the inotropic response to neural stimulation.

MET did not prolong the inotropic (Fig. 4) or chronotropic (Fig. 6) responses to sympathetic stimulation, whereas COC did prolong the cardiac responses substantially. This suggests that the neuronal uptake mechanisms are much more important than the extraneuronal mechanisms for the removal of the neurally released NE in the heart. Langer and Rubio (1973) have suggested that the relatively greater importance of neuronal over extraneuronal uptake mechanisms applies to structures, such as the heart, that have relatively narrow synaptic gaps.

In certain tissues, at least, there appears to be a dynamic balance between the two uptake processes for the removal of neurally released NE. When one uptake process is suppressed, the other process becomes more active. Hughes (1972) measured the output of NE evoked by electrical field stimulation in isolated portal vein and vas deferens preparations from the rabbit. Corticosterone, an extraneuronal uptake blocker, increased the NE output by 30-40% when it was given alone, whereas it caused a 300% increase in output in tissues pretreated with COC. When the order of drug administration was reversed, COC caused a much greater increase in NE output in tissues pretreated with corticosterone than in tissues not so pretreated. Similarly, Almgren and Jonason (1974) observed a significant interaction between the two uptake mechanisms in the rat salivary gland. At NE dose levels below 1 \( \mu \)g, NMN had no detectable influence on the secretory response to NE. However, after neuronal uptake blockade, the secretory response to NE was augmented significantly after extraneuronal uptake blockade with NMN. Conversely, Junstad et al. (1973) did not find an appreciable interaction between the neuronal and extraneuronal uptake mechanisms in the isolated rabbit heart. NMN increased the output of NE by 26% in response to sympathetic nerve stimulation, whereas desipramine, a neuronal uptake blocker, increased it by 106%. The combination of these two agents raised the NE output by only 71% above the control level, however. A similar phenomenon probably prevailed in our experiments. It is apparent from Figure 3 (middle panel) that the contractile force responses to anodal stimulation after the combination of COC and MET (\( P_3 \), groups MC and CM) were less than when either blocking agent was given alone (\( P_2 \), same groups).

Our data obtained in vivo are compatible with those of Junstad et al. (1973) obtained in isolated heart preparations. Such results suggest that, in the heart, there is no appreciable interaction between the neuronal and extraneuronal uptake mechanisms. The combined effects of COC and MET on
the magnitudes or durations of the inotropic and chronotropic responses to sympathetic stimulation in our experiments were no greater than the sums of the individual effects (Figs. 3–6).

It is concluded from the present study that the uptake processes for NE are important determinants of the cardiac responses. The neuronal uptake process appears to predominate in the inactivation of the neuronally released NE in the heart, whereas the extraneuronal uptake process seems to play a relatively minor role. The interaction between the neuronal and extraneuronal uptake mechanisms are not pronounced in the heart, although they appear to be very important in certain other tissues. Perhaps the narrowness of the synaptic clefts in cardiac tissue accounts for this prepotence of the neuronal uptake mechanism over the extraneuronal uptake process and for the absence of any appreciable interaction between these two processes.

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