Role of cholinergic receptors and cholinesterase activity in hemodynamic responses to cocaine in conscious rats

MARK M. KNUEPFER AND QI GAN
Department of Pharmacological and Physiological Science, St. Louis University School of Medicine, St. Louis, Missouri 63104

Knuefer, Mark M., and Qi Gan. Role of cholinergic receptors and cholinesterase activity in hemodynamic responses to cocaine in conscious rats. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R103–R112, 1999.—It has been suggested that toxicity to cocaine is related to the relative rate of cocaine metabolism by cholinesterases and to activation of cholinergic receptors either directly or by reflex mechanisms. We examined these possibilities by altering cholinesterase activity and blocking cholinergic receptors in rats prone or resistant to cocaine-induced cardiovascular toxicity. Rats were instrumented with a pulsed Doppler flow probe on the ascending aorta for measurement of cardiac output and cannulated for arterial pressure and heart rate determination. In conscious rats, cocaine (5 mg/kg iv) elicited pressor responses and a delayed bradycardia but cardiac output and systemic vascular resistance responses varied greatly between rats. Pretreatment with the nonspecific cholinesterase inhibitors physostigmine (0.1–0.2 mg/kg) or neostigmine (0.1 mg/kg) reduced the pressor response by diminishing the increase in systemic vascular resistance. In contrast, inhibition of cocaine metabolism with the selective plasma cholinesterase inhibitor tetraisopropyl pyrophosphoramide (0.5 mg/kg) or increasing cholinesterase activity with human butyryl cholinesterase (9.9 mg/kg iv) did not alter hemodynamic responses to cocaine. Administration of atropine methyl bromide (0.5–1 mg/kg iv) alone or with physostigmine to prevent the cholinomimetic effects of physostigmine reduced the cocaine-induced decrease in cardiac output noted in some animals. These data suggest that the cocaine-induced decrease in cardiac output observed in some rats is, at least in part, dependent on activation of muscarinic receptors. In addition, the rate of cocaine metabolism is not critical for the initial hemodynamic responses to cocaine in conscious rats.

COCAINE EVOKES ADVERSE cardiovascular responses in some individuals, including myocardial ischemia, arrhythmias, sudden cardiac death, and coronary vasoconstriction (13, 25, 31, 38). As yet, factors determining individual predisposition to cocaine-induced toxicity are not well understood. We proposed a model for varying sensitivity and toxicity in rats that is related to the wide variability of cardiac output and systemic vascular resistance responses to cocaine in conscious rats (3, 4, 19, 20). In some rats, cocaine administration elicited a decrease in cardiac output and an increase in systemic vascular resistance of at least 80%, whereas cocaine consistently elicited little change or an increase in cardiac output and smaller increases in systemic vascular resistance in the remaining rats (3). After dividing these into two groups to facilitate the analysis of possible differences (3, 4), we termed the rats vascular responders and mixed responders, respectively, in the present study. Repeated cocaine administration produces hypertension and more severe cardiomyopathies in vascular responders compared with mixed responders (4, 20). Acute behavioral stress produces similar variable hemodynamic response patterns both in rats (21) and in humans (6, 42). Humans described as vascular responders to acute stress have been described as susceptible to stress-induced hypertension (42) and heart disease (6). Therefore, we suggested that cocaine (and other psychoactive agents) and stress elicit similar cardiovascular responses in individuals and that the rat may provide a model for determining the causes of varying cardiovascular sensitivity to cocaine-induced cardiotoxicity in humans (3, 20).

Others have suggested that variations in cocaine metabolism contribute to individual sensitivity to cocaine-induced toxicity (10, 14, 35) because plasma cholinesterase activity, the primary mechanism by which cocaine is metabolized to less active compounds in humans and rats (1, 15, 32, 39), varies substantially among individuals. Therefore, humans with lower plasma cholinesterase activity are more likely to experience life-threatening toxicity to cocaine compared with those with greater activity (10). It has been reported that nonselective inhibition of cholinesterase activity with physostigmine (0.3 mg/kg ip) reduces cocaine-induced toxicity in rats (46). Selective inhibition of plasma cholinesterases with tetrakisopropyl pyrophosphoramide (iso-OMPA) is reported to enhance toxicity to cocaine in conscious mice (12), reduce toxicity in anesthetized rats (17), and not alter toxicity in anesthetized pigs (16). Butyryl cholinesterase is the primary mechanism by which cocaine is hydrolyzed (8). Cholinesterase activity, enhanced in rodents by administration of human butyryl cholinesterase or cholinesterase, resulted in reduced pressor and arrhythmogenic effects of cocaine and reduced convulsant activity and toxicity to cocaine (12, 27, 29). Therefore, several authors have suggested that varying cholinesterase activity may be responsible for variable sensitivity to cocaine and its toxicity.

Cholinergic receptors have also been implicated in the cardiovascular responses to cocaine. Graham et al. (9) reported that bilateral vagotomy and atropine administration prevented the bradycardia and increase in right atrial pressure but not the pressor response to cocaine in chloralose-urethan-anesthetized dogs. Wilkerson (45) reported that atropine alone augmented the...
small pressor response to cocaine without affecting heart rate or coronary vascular resistance in pentobarbital sodium-anesthetized dogs. From these data, the author concluded that atropine may enhance cocaine-induced toxicity. Tella and co-workers (40) reported that atropine attenuated an idiosyncratic profound bradycardia noted after cocaine administration in a conscious squirrel monkey. Shannon and co-workers (36) demonstrated that atropine methyl bromide enhanced the cocaine-induced increases in contractility and coronary blood flow in conscious dogs. We reported that atropine methyl bromide enhanced cocaine-induced hindquarters vasodilation and reduced the increase in systemic vascular resistance in conscious rats (18). Therefore, cocaine appears to mediate some hemodynamic responses by activation of peripheral muscarinic receptors.

The present study was performed to determine how alterations in cholinesterase and muscarinic receptor activity change the hemodynamic responses to cocaine. To examine the effects of cocaine metabolism on toxicity, we compared the effects of selective inhibition of plasma cholinesterase using iso-OMPA with the effects of enhanced cholinesterase activity using human butyryl cholinesterase. We examined the effects of nonselective inhibition of cholinesterase activity using physostigmine because of its purported ability to reduce toxicity. We also examined the effects of a selective peripheral cholinomimetic, neostigmine. Finally, the effects of muscarinic receptor blockade both with and without physostigmine present were determined to address whether cocaine activates cholinergic receptors directly or through a parasympathomimetic action. The results suggest that cholinergic receptor activation, not altered cholinesterase activity, is responsible for the decrease in cardiac output noted in vascular responders.

MATERIALS AND METHODS

Animal preparation. All surgical and experimental procedures were approved by the St. Louis University Institutional Animal Care and Use Committee and followed guidelines described in the Guide for the Care and Use of Laboratory Animals. Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 280–420 g were surgically prepared under ketamine-xylazine anesthesia using aseptic techniques as previously described (3, 4, 18–22). A thoracotomy was performed and a miniaturized pulsed Doppler flow probe (Iowa Doppler Products, Iowa City, IA) filled with acoustic gel was sutured snugly on the ascending aorta. The thorax was closed and the lead wires brought subcutaneously to a socket on the skull. Rats were treated with cefazolin (10 mg/kg im, once daily for 3 days to reduce the risk of sepsis) and buprenorphine (0.06 mg sc) and allowed to recover for a minimum of 10 days. Rats with poor or varying velocity signals or that did not recover normal motor behavior within 24 h were euthanized with an overdose of pentobarbital sodium. Seven to 10 days after recovery, rats were anesthetized with methoxyflurane for implantation of femoral arterial and venous cannulas filled with 15 mg/ml cefazolin. In a separate group of rats (n = 8) used to test potential toxicity of higher doses of some agents, only femoral arterial and venous cannulas were implanted. One to 3 days after cannulation, all rats were acclimated in a Plexiglas cage for 6 h. On the following day, rats were placed in the same cage for 2 h before experimentation was begun.

Experimental procedure. The procedures employed in these experiments have been described in detail (3, 4, 18–22). During and after the daily 2-h acclimation period, arterial pressure, heart rate, and aortic blood flow were monitored continuously. Rats were studied for up to 2 wk. A single dose of cocaine hydrochloride (5 mg/kg iv, infused over 45 s) was utilized alone or after pretreatment with another agent. Lower doses of cocaine and more rapid administration also elicited variable cardiac output responsiveness in rats (3), but the responses are less reproducible. Therefore, this dose was utilized because it consistently elicits specific cardiac output responses in individual rats, allowing classification of vascular and mixed responders without producing overt toxicity. Cocaine was administered twice daily with a minimum dosing interval of 4 h. Typically, cocaine was given alone in the morning and was given again 10 min after pretreatment in the afternoon. We have not observed significant tachyphylaxis of cardiovascular responses to cocaine when given alone in the morning and afternoon nor when given twice daily for up to 6 days (4). All experiments were conducted between the hours of 9 AM and 4 PM in a quiet room.

The contribution of agents enhancing or reducing cholinesterase activity was examined. To determine the causes of toxicity noted by others with physostigmine pretreatment, the nonselective cholinesterase inhibitor physostigmine (0.1–1 mg/kg) was administered. Physostigmine inhibits butyryl cholinesterase activity with a Kᵢ of 4.5 × 10⁻¹⁹ M⁻¹·min⁻¹ (27). Responses to physostigmine were compared with those with neostigmine (0.1 mg/kg) due to the inability of the latter drug to cross the blood-brain barrier. This dose of neostigmine was used in a related study (46). In addition, the specific plasma cholinesterase inhibitor iso-OMPA was administered intravenously in doses of 0.5–2 mg/kg. Plasma cholinesterase activity is reduced by 78% (11) to 92% (17) with iso-OMPA (1 mg/kg sc). In addition, iso-OMPA (1–2 mg/kg) inhibits butyryl cholinesterase activity selectively (24, 27). Butyryl cholinesterase activity was selectively enhanced by administration of human butyryl cholinesterase (9.9 mg/kg). A smaller dose of butyryl cholinesterase (8 mg/kg iv) elicits a leftward shift in the dose-response curve to acetylcholine (27). Due to the long-lasting effects on cholinesterase activity and cocaine responsiveness after treatment with this enzyme (27), rats were not retested after treatment with butyryl cholinesterase for at least 3 days.

To discriminate the effects of physostigmine on peripheral muscarinic cholinergic neurotransmission, the effects of atropine methyl bromide (0.5 and 1 mg/kg) alone and in combination with physostigmine were investigated. A dose of 0.5 mg/kg atropine methyl bromide prevents the bradycardia elicited by 0.1 µg acetylcholine in rats (22).

Eight rats were treated with physostigmine, atropine methyl bromide, and the combination of the two agents on different days. Of the remaining rats, 13 were tested with two antagonists (on different days) and 1 was examined in three different experimental protocols. In all cases, control responses to cocaine were repeated before each pretreatment regimen.

Materials. Materials used included iso-OMPA, neostigmine sulfate, and atropine methyl bromide from Sigma Chemical (St. Louis, MO), cocaine hydrochloride from the National Institute on Drug Abuse, physostigmine sulfate from Research Biochemicals (Natick, MA), and human butyryl cholinesterase from Shire Laboratories (Rockville, MD). Drugs used during surgery and recovery included pentobarbital sodium.
RESULTS

Conscious rats (n = 56) had a mean arterial pressure of 117.6 ± 1.3 mmHg, a heart rate of 394 ± 5 beats/min, and an ascending aortic velocity signal of 10.2 ± 0.2 kHz shift. Cocaine administration (5 mg/kg iv) elicited pressor responses and variable changes in cardiac output and systemic vascular resistance (Fig. 1). Each rat received cocaine alone several times (3–13 trials, mean = 6.9 ± 0.4 trials) to classify hemodynamic responsivity and provide control responses before pretreatments. Individual rats (n = 41) were designated vascular responders if the mean decrease in cardiac output was >8% (mean = −14.6 ± 1.4%). The remaining rats, classified as mixed responders, had smaller decreases or increases in cardiac output (mean = 10.8 ± 3.0%). These changes were significantly different from one another and from baseline values. The resting arterial pressure and heart rate and the mean ascending aortic flow signal were not different between groups. Figure 1 depicts the time course of cardiovascular responses to cocaine alone in vascular and mixed responders. No differences in resting arterial pressure, heart rate, or aortic flow were identified between various groups except where noted (Table 1).

Effects of physostigmine. The nonspecific cholinesterase inhibitor physostigmine (0.1–0.2 mg/kg iv) elicited increases in arterial pressure and systemic vascular resistance and decreases in heart rate and cardiac output that were still present 10 min after drug administration (Table 2). There were no differences in the hemodynamic effects elicited by 0.1 mg/kg physostigmine and those evoked by 0.2 mg/kg physostigmine. In initial experiments with rats not instrumented for cardiac output determination, treatment with 0.5 mg/kg physostigmine elicited a profound bradycardia and a pressor response followed by a depressor response and death unless atropine (0.5 mg/kg) was administered (2 of 5 rats).

Cocaine (5 mg/kg) was administered after physostigmine (0.1 and 0.2 mg/kg, n = 14 and 9, respectively). Because hemodynamic responses were similar at each dose, the data were combined in Fig. 2. Physostigmine pretreatment reduced the pressor responses to cocaine due to a smaller increase in systemic vascular resistance (Fig. 2). Alternatively, these reductions may have resulted from changes in baseline values for these
Table 1. Baseline hemodynamic variables

<table>
<thead>
<tr>
<th></th>
<th>Mean Arterial Pressure, mmHg</th>
<th>Heart Rate, beats/min</th>
<th>Cardiac Output, kHz shift</th>
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<td></td>
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<tr>
<td>Physostigmine</td>
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<tr>
<td>MR</td>
<td>7 118 ± 5</td>
<td>386 ± 12</td>
<td>10.2 ± 0.8</td>
</tr>
<tr>
<td>VR</td>
<td>16 119 ± 2</td>
<td>385 ± 5</td>
<td>10.4 ± 0.2</td>
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<tr>
<td>Neostigmine</td>
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<tr>
<td>VR</td>
<td>7 114 ± 4</td>
<td>430 ± 15</td>
<td>11.0 ± 0.3</td>
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<td>Iso-OMPA</td>
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<tr>
<td>MR</td>
<td>6 117 ± 4</td>
<td>368 ± 8</td>
<td>9.1 ± 0.5</td>
</tr>
<tr>
<td>VR</td>
<td>9 124 ± 4</td>
<td>383 ± 8</td>
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<tr>
<td>VR</td>
<td>10 122 ± 3</td>
<td>405 ± 9</td>
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<td>AMB</td>
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<tr>
<td>MR</td>
<td>5 108 ± 2</td>
<td>409 ± 28</td>
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</tr>
<tr>
<td>VR</td>
<td>11 121 ± 3</td>
<td>376 ± 7</td>
<td>10.3 ± 0.7</td>
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<td>Physostigmine + AMB</td>
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<tr>
<td>VR</td>
<td>7 119 ± 1</td>
<td>371 ± 7</td>
<td>10.3 ± 0.3</td>
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</table>

Values are means ± SE; n = no. of animals. MR, mixed responders; VR, vascular responders; iso-OMPA, tetraisopropyl pyrophosphoramide; AMB, atropine methyl bromide. Some rats were used in more than 1 experimental paradigm, as described in METHODS.

*Significant difference from corresponding value in mixed responders (P < 0.05).

parameters (Table 2). Physostigmine enhanced the decreases in heart rate (Fig. 2). The maximum change in cardiac output and associated change in systemic vascular resistance were still different between vascular and mixed responders (Fig. 3).

Effects of neostigmine. Because only one of eight rats studied with neostigmine was a mixed responder, only the data from the seven vascular responders are reported here. Neostigmine (0.1 mg/kg iv) caused a decrease in heart rate and cardiac output 10 min after dosing (Table 2). Subsequent administration of cocaine resulted in a significant reduction in the peak pressor response and the peak increase in systemic vascular resistance without altering cardiac output responses (data not shown). At the time of the maximum increase in cardiac output, there was a significant reduction in the pressor response and the increase in systemic vascular resistance (Fig. 3).

Effects of iso-OMPA. The resting cardiac output was significantly lower in mixed responders compared with vascular responders in the subset of rats (n = 15) studied with iso-OMPA (Table 1). Ten minutes after administration of the selective plasma cholinesterase inhibitor iso-OMPA (0.5 mg/kg), hemodynamic parameters were not different from baseline values (Table 2). Iso-OMPA pretreatment did not affect hemodynamic responses to cocaine (Fig. 4). Differences in cardiac output at the time of the peak cardiac output response between vascular and mixed responders were still present after iso-OMPA (Fig. 3).

Higher doses of iso-OMPA (1 and 2 mg/kg, n = 2 and 8, respectively) produced a brief pressor response initially and caused a small but significant decrease in the peak pressor response to cocaine in rats instrumented with arterial and venous canulas only (data not shown).

Effects of human butyryl cholinesterase. Using butyryl cholinesterase isolated from human plasma, we examined the effects of selectively enhancing the rate of cocaine metabolism. Administration of human butyryl cholinesterase (9.9 mg/kg) enhances cocaine metabolism and degradation in rats (29). In the present study, this dose did not alter arterial pressure, heart rate, cardiac output, or systemic vascular resistance 10 min after administration (Table 2) when given alone to vascular responders (n = 10). Subsequent administration of cocaine (5 mg/kg) resulted in similar hemodynamic responses as observed before treatment with butyryl cholinesterase (Figs. 3 and 5).

Effects of atropine methyl bromide. The effects of atropine methyl bromide were examined to determine the contribution of muscarinic receptors. In this subset of rats, mixed responders had significantly lower resting arterial pressure compared with vascular responders (P = 0.032, Table 1). Atropine methyl bromide pretreatment (0.5 or 1 mg/kg) did not alter arterial pressure in either group but did elicit a significant increase in heart rate (Table 2). In vascular responders, atropine methyl bromide alone evoked an increase in cardiac output and a decrease in systemic vascular resistance. These changes were not observed in mixed responders (Table 2).

Atropine methyl bromide pretreatment did not affect the pressor response to cocaine (Fig. 6) administration (5 mg/kg), but reduced the bradycardic response in all rats, possibly because of a shift in the heart rate after atropine methyl bromide (Table 2). Atropine methyl bromide pretreatment prevented the decrease in cardiac output and reduced the increase in systemic vascular resistance in vascular responders (Fig. 3). The change in systemic vascular resistance responses was due, in part, to a reduction in vascular resistance elicited by atropine methyl bromide alone (Table 2).

Table 2. Change in baseline values

<table>
<thead>
<tr>
<th></th>
<th>Arterial Pressure, mmHg</th>
<th>Heart Rate, beats/min</th>
<th>Cardiac Output, kHz shift</th>
<th>Systemic Vascular Resistance, kHz shift</th>
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<td>Physostigmine</td>
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<tr>
<td>MR</td>
<td>7 21.1 ± 2.1*</td>
<td>-41 ± 13</td>
<td>-11.3 ± 2.6*</td>
<td>33.3 ± 4.0*</td>
</tr>
<tr>
<td>VR</td>
<td>16 15.1 ± 2.5</td>
<td>-34 ± 6</td>
<td>-5.9 ± 2.0*</td>
<td>22.8 ± 3.4*</td>
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<tr>
<td>Neostigmine</td>
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<tr>
<td>VR</td>
<td>7 -2.3 ± 2.8</td>
<td>-34 ± 10</td>
<td>-7.2 ± 1.4*</td>
<td>5.9 ± 2.5</td>
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<tr>
<td>Iso-OMPA</td>
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<tr>
<td>MR</td>
<td>6 -2.2 ± 1.6</td>
<td>-8 ± 3</td>
<td>-9 ± 1.0</td>
<td>-0.7 ± 1.6</td>
</tr>
<tr>
<td>VR</td>
<td>9 -2.7 ± 1.6</td>
<td>6 ± 9</td>
<td>0.2 ± 1.1</td>
<td>-2.4 ± 1.8</td>
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<td>Butyryl cholinesterase</td>
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<tr>
<td>VR</td>
<td>10 -2.8 ± 2.5</td>
<td>-4 ± 5</td>
<td>-2.5 ± 2.1</td>
<td>0.6 ± 1.8</td>
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<tr>
<td>AMB</td>
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<tr>
<td>MR</td>
<td>5 -0.4 ± 2.2</td>
<td>55 ± 16</td>
<td>0.5 ± 2.3</td>
<td>-0.4 ± 2.9</td>
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<tr>
<td>VR</td>
<td>11 -0.9 ± 1.5</td>
<td>76 ± 9*</td>
<td>4.0 ± 1.5*</td>
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<tr>
<td>VR</td>
<td>7 21.7 ± 2.6*</td>
<td>49 ± 11</td>
<td>-1.1 ± 2.4</td>
<td>20.5 ± 4.2*</td>
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Values are means ± SE; values were obtained 10 min after treatment (immediately before cocaine administration). *Significant difference from control values (P < 0.05).
Effects of physostigmine and atropine methyl bromide. To examine the effects of physostigmine on cocaine metabolism only, atropine methyl bromide (0.5 mg/kg) was administered, followed by physostigmine (0.2 mg/kg). Ten minutes after administration of physostigmine, arterial pressure, heart rate, and systemic vascular resistance were elevated in seven vascular responders tested (Table 2).

Subsequent administration of cocaine (5 mg/kg iv) reduced the peak pressor response due to a smaller increase in systemic vascular resistance (Fig. 7). As seen with atropine alone, the decrease in cardiac output was prevented during the modest sustained pressor response (1, 3, and 5 min). The maximum decrease in cardiac output and the associated increase in systemic vascular resistance were reduced also (Fig. 3).

DISCUSSION

The data indicate that some of the hemodynamic responses to cocaine are mediated by muscarinic receptor activation. Atropine methyl bromide prevented the decrease in cardiac output noted in vascular responders. This can be explained either by 1) direct activation of muscarinic receptors or 2) indirect responses due to cocaine-induced vagal reflexes. There is evidence that cocaine acts directly on cholinergic receptors. Shannon et al. (36) studied cardiac responses in conscious dogs and reported that atropine methyl bromide pretreatment enhanced the cocaine-induced increase in cardiac contractility and cardiac oxygen consumption and reduced the increase in coronary vascular resistance. Sharkey et al. (37) demonstrated that (-)-cocaine has affinity for muscarinic receptors. Miao et al. (30) re-
ported that cocaine has a negative inotropic effect on ferret ventricular tissue that is due, in part, to activation of muscarinic receptors. These data indicate that cocaine may affect cholinergic receptors directly.

Alternatively, vagal activation may be responsible for cocaine-induced muscarinic activation. Cocaine elicits a substantial bradycardia in rats that has been proposed to result, in part, from baroreflex-mediated increases in vagal activity to the heart (9, 23, 41). In the present study, no attempt was made to assess baroreflex function despite its possible contribution to these responses. As suggested by Shannon et al. (36), the increase in vagal activity could directly inhibit release of adrenergic neurotransmitters in the myocardium (26, 33) and in other organs (2, 47). We reported that cocaine elicited a burst in renal sympathetic nerve activity that was particularly prominent in vascular responders (5). Others reported that after cocaine or sympathectomy, atropine was capable of enhancing contractility responses to exogenous norepinephrine (43). We reported that beta-adrenergic blockade exacerbates the decrease in cardiac output in vascular responders and makes mixed responders respond with a decrease in cardiac output (4). Likewise, Shannon et al. (36) demonstrated that propranolol enhanced and atropine methyl bromide reduced the cocaine-induced increase in coronary vascular resistance in conscious dogs. These data indicate that muscarinic and adrenergic receptors mediate some cardiovascular responses to cocaine in an opposing manner. If muscarinic receptor

Fig. 4. Responses to cocaine (5 mg/kg iv) before and after pretreatment with iso-OMPA (0.5 mg/kg iv) in mixed and vascular responders (n = 6 and 9, respectively). Data are presented as described for Figs. 1 and 2.

Fig. 5. Responses to cocaine (5 mg/kg iv) before and after pretreatment with butyryl cholinesterase (9.9 mg/kg iv) in vascular responders (n = 10) only. Data are presented as described for Figs. 1 and 2.
activation reduces catecholamine release as suggested (26, 33), muscarinic antagonists would reduce the vagal influence on sympathetic nerve terminals, thereby enhancing norepinephrine release and reducing the decrease in cardiac output.

Wilkerson (45) reported that atropine administration enhanced pressor responses to cocaine in pentobarbital sodium-anesthetized dogs, leading him to the conclusion that muscarinic antagonists could increase toxicity. This interpretation is suspect for several reasons. First, anesthesia has profound effects on hemodynamic responses to cocaine (18, 44). In addition, atropine sulfate blocks muscarinic receptors both systemically and in the central nervous system (CNS). Finally, it has not been demonstrated that the magnitude of the pressor response is directly related to cocaine-induced toxicity. In fact, Witkin et al. (46) reported that atropine pretreatment did not alter toxicity to cocaine in conscious rats. This was determined by administering high doses of cocaine (60–120 mg/kg) intraperitoneally. The discrepancy between the cardiovascular studies and this toxicity study may be due to the inability of low doses of cocaine, used in cardiovascular studies, to predict toxicity or to the possibility that high doses of cocaine do not always replicate human toxicity. Indeed, several authors have noted that humans experiencing cardiovascular complications or sudden cardiac death after cocaine use have variable and often relatively low plasma levels of cocaine, suggesting that individuals vary widely in their sensitivity to cocaine-induced cardiotoxicity (13, 31, 38) as we noted in rats (20).

Fig. 6. Responses to cocaine (5 mg/kg iv) before and after pretreatment with atropine methyl bromide (0.5 or 1 mg/kg iv) in vascular (n = 11) and mixed responders (n = 5). Data are presented as described for Figs. 1 and 2. *Significant differences due to drug pretreatment as determined by ANOVA.

Fig. 7. Responses to cocaine (5 mg/kg iv) before and after pretreatment with atropine methyl bromide (0.5 or 1 mg/kg iv) and physostigmine (0.1 mg/kg iv) in vascular responders (n = 7) only. Data are presented as described for Figs. 1 and 2. *Significant differences due to drug pretreatment as determined by Student's t-test (peak value) and ANOVA (1-, 3-, and 5-min values).
Several reports have suggested that varying cholinesterase activity may affect the predisposition toward cocaine-induced toxicity in individuals (10, 11, 14, 35). Witkin et al. (46) reported that a lower dose (0.3 mg/kg ip) of the nonselective cholinesterase inhibitor physostigmine reduced toxicity whereas a higher dose (1 mg/kg ip) enhanced toxicity. Our results demonstrate that intravenous physostigmine (0.1–0.2 mg/kg) alters hemodynamic responses to cocaine possibly by changing the resting values of several parameters. For example, rats had a reduced pressor response due to smaller increases in systemic vascular resistance to cocaine after physostigmine, possibly resulting from higher arterial pressure and systemic vascular resistance (Table 2). We obtained similar results in five vascular responders treated with cocaine (1 mg/kg iv) after pretreatment with 0.1 mg/kg physostigmine (unpublished data). The vascular effects resulting in a pressor response to physostigmine may result from the ability of physostigmine to stimulate nicotinic receptors (28). At a higher dose (0.5 mg/kg iv), physostigmine alone was lethal.

Physostigmine is not specific because it inhibits both circulating and neuronal cholinesterases. In addition, it acts in the rostral ventrolateral medulla to enhance cholinergic receptor activation, increasing arterial pressure (9, 34). Therefore, we used neostigmine, a quaternary anticholinesterase that does not readily cross the blood-brain barrier, to differentiate possible CNS effects of physostigmine. Neostigmine pretreatment resulted in reductions in cocaine-induced increases in arterial pressure and systemic vascular resistance as noted with physostigmine. Witkin and co-workers (46) reported that 0.1 mg/kg neostigmine significantly attenuated the lethality of cocaine at a high dose (100 mg/kg) and potentiated the lethality of cocaine at 0.3 mg/kg neostigmine. The similarity between the effects of physostigmine and neostigmine to reduce pressor responses and increases in systemic vascular resistance suggests that the results with cholinesterase inhibitors reflect differences in effects on peripheral muscarinic receptors. We cannot deduce whether cocaine metabolism is impaired because cocaine levels were not measured. In any case, a reduction in cocaine metabolism would be expected to enhance hemodynamic responses, not reduce them.

The selective butyryl cholinesterase inhibitor iso-OMPA (24) has been used to differentiate the effects of nonspecific cholinesterase inhibitors on cocaine metabolism with the effects on parasympathetic nerves. Kambam and co-workers (17) reported that 1 mg/kg iso-OMPA protects rats from the acute lethality of cocaine but also noted that iso-OMPA (2 mg/kg) does not alter toxicity to infusions of cocaine in the pig (16). In contrast, Hoffman et al. (11) reported that 1 mg/kg iso-OMPA enhances toxicity to cocaine in mice. We observed that iso-OMPA (0.5–2 mg/kg) had very little effect on cocaine-induced hemodynamic responses. The differences may be related to genetic variations in butyryl cholinesterase activity.

Dretchen and co-workers (27) reported that administration of human butyryl cholinesterase (7.8 mg/kg iv) produced no effects on cardiovascular parameters despite causing a 100-fold rightward shift in the dose-response curve to exogenous acetylcholine in conscious rats. The same dose reduced the hypertensive and arrhythmogenic effects of cocaine and reduced the associated hyperactivity (27, 29). Butyryl cholinesterase administration resulted in a three- to fourfold increase in the lethal dose of cocaine in anesthetized rats (27) and reduced both plasma and brain concentrations of cocaine (29). Therefore, the enhanced ability to metabolize cocaine reduces the toxic consequences. In the present study using lower, nontoxic doses, we did not note differences in the initial cardiovascular responses to cocaine after pretreatment of vascular responders with human butyryl cholinesterase. Considering the long duration of action of butyryl cholinesterase (27) and the often delayed toxicity noted in humans (13, 31), it is possible that this enzyme may still have a beneficial effect on reducing toxicity as suggested by others (27, 29). It is also possible that the maximum hemodynamic responses we recorded in the first 1–2 min are not altered by enhancing cocaine metabolism because they occur before the majority of the metabolic degradation occurs. In contrast, our slower rate of infusion (45 s) results in lethality only at higher doses (8–10 mg/kg). In addition, we were unable to note an enhancement in this response after inhibition of butyryl cholinesterase with iso-OMPA. Therefore, the initial hemodynamic responses may be affected by the rate of administration and the likelihood of toxicity.

More recently, Hoffman and co-workers (12) reported that administration of human cholinesterase to mice protected them from the lethal effects of cocaine. These data suggest that the effects on plasma cholinesterases may also be important. Our data suggest that the pressor and vascular responses are enhanced by cholinomimetics. We propose that this is likely due to an action on muscarinic receptors rather than on cocaine metabolism. This proposal is based on arguments presented earlier, although this could be verified by direct assessment of plasma cocaine and metabolite levels.

Witkin and co-workers (46) reported that intraperitoneal atropine does not alter the beneficial effects of low-dose physostigmine on cocaine-induced lethality. In the present study, administration of atropine methyl bromide reduced the decrease in cardiac output and the increase in systemic vascular resistance in vascular responders. Coadministration of physostigmine and atropine methyl bromide reduced the fall in cardiac output and increase in systemic vascular resistance in vascular responders similar to the effects of atropine.
methyl bromide alone. The pressor response to cocaine was reduced by this combination as with physostigmine alone, although the change may have been due to a shift in baseline arterial pressure.

In summary, selective enhancement or inhibition of cocaine metabolism does not appear to alter hemodynamic responsiveness to cocaine in conscious rats. In contrast, the depression in cardiac function noted in some rats by a decrease in cardiac output is mediated, at least in part, by activation of muscarinic receptors. The muscarinic receptors contribute to both cardiac and peripheral vascular resistance responses. We suggest that this may result from muscarinic receptor-induced inhibition of catecholamine release. The specific mechanism remains to be determined.

Perspectives

Several lines of evidence suggest that humans have characteristic response patterns to behavioral stress and that the response patterns may provide the means to predict individuals at greater risk for cardiovascular disease (6, 42). Likewise, humans appear to vary widely in their predisposition to cocaine-induced cardiotoxicity (10, 13, 31, 38). Our studies suggest that the rat may be a model for varying hemodynamic responsiveness (3, 4, 19, 20) and that the responsiveness to psychostimulants correlates with responses evoked by behavioral stress (21). The present study provides evidence that muscarinic receptors are important in this varying responsiveness. This, in turn, implicates muscarinic receptors in the development of cardiovascular disease. The results suggest that additional studies into the role of peripheral cholinergic receptors, muscarinic regulation of catecholamine release, and, possibly, reflex parasympathetic responsiveness may provide important insights into the means by which stress or psychoactive agents contribute to the development of hypertension and heart disease.

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Address for reprint requests: M. M. Knuepfer, Dept. of Pharmacological and Physiological Science, St. Louis Univ. School of Medicine, 1402 S. Grand Blvd., St. Louis, MO 63104.

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