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Muscarinic cholinergic and β-adrenergic contribution to hindquarters vasodilation and cardiac responses to cocaine

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MAP, mean arterial pressure, CO, cardiac output; HqR, hindquarters vascular resistance; HR, heart rate, SV, stroke volume; SVR, systemic vascular resistance.

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Abstract

Cocaine produces a pressor response associated with an initial hindquarters vasoconstriction followed by a prolonged vasodilation in conscious rats. Propranolol pretreatment prevented the vasodilation and enhanced the pressor response whereas atropine methylbromide pretreatment reduced the increase in systemic vascular resistance. We studied the role of selective muscarinic and β-adrenoceptor antagonists on responses to cocaine in rats with an increase in systemic vascular resistance to cocaine (vascular responders). Arterial blood pressure and ascending aortic and distal descending aortic blood flow using pulsed Doppler flowmetry were measured. In conscious rats, cocaine (5 mg/kg, i.v.) elicited consistent pressor responses but variable systemic and hindquarters vascular resistance responses that were directly correlated suggesting that skeletal muscle resistance responses comprise an important component of systemic vascular resistance. ICI 118,551 (0.5 mg/kg, iv) pretreatment prevented the hindquarters vasodilation enhancing the increase in systemic vascular resistance and the pressor response while further depressing the cardiac output response similar to the effects of propranolol. Atenolol (1 mg/kg) pretreatment attenuated the stroke volume and cardiac output response while enhancing the increase in systemic vascular resistance without affecting the hindquarters responses. In contrast, the M_2 antagonist, methoctramine (0.3 mg/kg) pretreatment had similar effects as atropine in reducing the decrease in cardiac output by reducing the increase in systemic vascular resistance whereas the M_1 antagonist, pirenzipine (0.02 mg/kg), did not alter responses. Therefore, the cocaine-induced pressor response is ameliorated by β_2-adrenoceptor mediated skeletal muscle vasodilation whereas the decrease in cardiac output and the increase in systemic vascular resistance are dependent on M_2-cholinoceptor activation.

Skeletal muscle blood flow is regulated largely by the sympathoadrenal system and is, therefore, particularly sensitive to modulation by the CNS. Behavioral stress produces hindquarters vasodilation by a β_2-adrenergic mechanism (Herd, 1991; Kirby and Johnson, 1990). The resulting reduction in systemic vascular resistance has been suggested to have profound effects on the ability of a stressor to evoke an increase in cardiac output (Herd, 1991). We have shown that acute hemodynamic responses to behavioral stress are similar to responses elicited by cocaine (Knuepfer et al., 2001). Several investigators have suggested that CNS-mediated sympathoexcitation is important in mediating the cardiovascular effects of cocaine in animals (Branch and Knuepfer, 1994b; Chiueh and Kopin, 1978; Kiritsy-Roy et al., 1990; Knuepfer and Branch, 1992; Tella et al., 1993; Wilkerson, 1988) and humans (Jacobsen et al., 1997; Vongpatanasin et al., 1999). Cocaine administration elicits an increase in plasma catecholamines (Chiueh and Kopin, 1978; Kiritsy-Roy et al., 1990; Tella et al., 1993) to a similar extent as adrenal responses to behavioral stress. Ganglionic blockade reduces arterial pressure and heart rate responses to cocaine, in part, by attenuating the initial increase in mesenteric and hindquarters vascular resistances (Branch and Knuepfer, 1994a, Kiritsy-Roy et al., 1990; Knuepfer and Branch, 1992; Tella et al., 1993; Wilkerson, 1988). Therefore, cocaine and acute stress elicit a sympathoadrenal response.

After the initial peak pressor response to cocaine, the increase in arterial pressure appears to be reduced by a delayed hindquarters vasodilation despite continued mesenteric vasoconstriction (Knuepfer and Branch, 1992). The magnitude of the delayed skeletal muscle vasodilation is proportional to cardiac output responses and is prevented by propranolol or by adrenal demedullation and enhanced by pretreatment with prazosin (Branch and Knuepfer, 1992;

Knuepfer and Branch, 1992). Therefore, we proposed that the vasodilatory response to cocaine was mediated by β-adrenoceptors and epinephrine release and attenuated by α-adrenoceptor activation (Branch and Knuepfer, 1992; Knuepfer and Branch, 1992; Knuepfer and Mueller, 1999). It wasn't clear which β-adrenoceptor subtypes mediated responses to cocaine.

Cholinergic receptors also contribute to the hemodynamic responses to cocaine since atropine methylbromide (0.5-1 mg/kg) prevented the decreases in cardiac output and heart rate and enhanced the hindquarters vasodilation (Kiritsy-Roy et al., 1990; Knuepfer and Branch, 1992; Knuepfer and Gan, 1999). Several hypotheses have been suggested to explain the effects of muscarinic receptor blockade on hemodynamic responses to cocaine. It has been proposed that this effect was either due to a vagally-mediated baroreflex response (Kiritsy-Roy et al., 1990; Knuepfer and Branch, 1992), a direct action of cocaine on muscarinic receptors (Miao et al., 1996; Sharkey et al., 1988) or cholinergic alteration of catecholamine release from sympathetic nerve terminals (Lavallée et al., 1978; Muscholl, 1980; Shannon et al., 1993). The muscarinic receptor subtypes mediating hemodynamic responses have not been examined in detail.

In the present study, we hypothesized that β_2-adrenergic receptor activation in the hindquarters vasculature was responsible for cocaine-induced skeletal muscle vasodilation and, more importantly, for attenuating the increase in systemic vascular resistance elicited by cocaine. Moreover, we proposed that specific muscarinic receptor subtypes mediate the cardiac and skeletal muscle vascular responses to cocaine. We studied a subset of rats, named vascular responders, that have a pressor response due entirely to an increase in systemic vascular resistance since their cardiac output is reduced (Branch and Knuepfer, 1994a; Knuepfer and Mueller, 1999). Our results demonstrate a role for β_2-adrenoceptors in the cocaine-induced

hindquarters vasodilation and a role for M_2-receptors in evoking the decrease in cardiac output and stroke volume. Therefore, these receptor subtypes contribute to the hemodynamic actions of cocaine in different manners.

Methods

Animal Preparation:

All procedures followed the guidelines of the NIH (Guide for the Care and Use of Laboratory Animals) and were approved by the St. Louis University Institutional Animal Care and Use Committee. Under pentobarbital (45 mg/kg, i.p.) anesthesia, male Sprague-Dawley rats (n=18) weighing 280-360g were instrumented for determination of cardiac output. After tracheostomy, animals were mechanically ventilated using room air. A left thoracotomy was performed and a miniaturized pulsed Doppler flow probe (Iowa Doppler Products, Inc. Iowa City, IA) was placed around the ascending aorta for cardiac output estimation as described by our laboratory (Branch and Knuepfer, 1992, 1994a; Knuepfer et al., 1989, 1992). A second, smaller flow probe was placed on the descending aorta below the renal bifurcations after a mid-line laparotomy as described earlier (Branch and Knuepfer, 1992; Knuepfer et al., 1989; Knuepfer and Branch, 1992). The leads were tunneled subcutaneously to the skull where they were fixed with dental cement, and the rats treated with cefazolin (10 mg/kg, i.m.). Animals were allowed to recover for 1-2 weeks. Recovery to this and subsequent surgeries was monitored daily for at least 3 days. Recovery was considered complete if there was minimal loss in body weight (<10%), normal drinking and eating behavior and ambulation. If rats did not recover within 3 days, they were euthanized with pentobarbital (60-70 mg/kg, i.p.).

After recovery, rats were reanesthetized with a mixture of ketamine and xylazine (55 and 7 mg/kg, respectively, i.p.). The femoral artery and vein were cannulated with vinyl tubing that exited the skin between the scapulae. Rats were allowed to recover for 3 days before beginning testing.

Blood flow velocity was estimated continuously using a 20 MHz pulsed Doppler flowmeter (100 kHz sampling frequency, Department of Bioengineering, University of Iowa, Iowa City, IA). The velocity was measured as a change in incident frequency (kHz shift) and displayed as a voltage output on a Grass Model 7 chart recorder and stored electronically using WINDAQ software (DATAQ Instruments, Dayton, OH). Arterial pressure and heart rate were measured simultaneously.

Experimental Protocol:

After acclimation to the laboratory for a minimum of two hours, rats were tested in their home cage with cocaine (5 mg/kg, i.v., given over 45 s) twice daily with a minimum 3 hours between doses for a total of 4-6 trials per rat. The data from each trial in each rat were averaged and used to determine the hemodynamic response pattern in each rat. In previous studies, we noted that some rats had consistent increases in cardiac output in response to cocaine while others had decreases although the pressor responses were typically similar because the latter group, named vascular responders, had greater calculated increases in systemic vascular resistance (Branch and Knuepfer, 1994a; Knuepfer and Mueller, 1999). In the present study, we only examined the effects of these agents on vascular responders because the incidence of these rats is considerably greater using criteria defined earlier (Knuepfer and Mueller, 1999).

We recorded arterial pressure, heart rate, ascending aortic blood flow (as a measure of cardiac output) and descending aortic blood flow (as a measure of hindquarters blood flow) during the experimental trials. Arterial pressure and cardiac output changes in response to cocaine after saline or drug pretreatment were used to calculate systemic vascular resistance changes using Ohm's law (Knuepfer et al., 1989). Arterial pressure and hindquarters flow changes in response to saline or drug pretreatment were used to calculate changes in hindquarters

vascular resistance as described earlier (Knuepfer and Branch, 1992; Knuepfer et al., 1994). Changes in stroke volume were calculated using the formula that stroke volume is equivalent to the change in cardiac output divided by the change in heart rate (Knuepfer et al., 1989). All calculated responses were expressed as percent changes.

After observing responses to cocaine alone, saline or drug was administered intravenously 10 min before cocaine. Each drug pretreatment was compared to the hemodynamic responses obtained in the previous saline control injection. In this manner, each drug treatment had a control saline injection. Only one drug pretreatment was performed daily in each rat.

Drug pretreatments for investigating the role of β-adrenergic receptors included propranolol (1 mg/kg), atenolol (1 mg/kg), and ICI 118,551 (0.5 mg/kg). The drug doses were determined by testing with agonists, using previous data from our laboratory or from the reports of other laboratories. For example, we demonstrated that this dose of propranolol attenuated the depressor and heart rate responses to isoproterenol administration (Branch and Knuepfer, 1992). In several experiments (n=6), isoproterenol (0.1 μg/kg, i.v.) was administered before and 5 min after ICI 118,551 administration to determine the extent of β-adrenoceptor blockade. ICI 118,551 pretreatment prevented the isoproterenol-induced hindquarters vasodilation (-38.5 +/- 4.5% before vs. 1.1 +/- 6.3% after ICI 188,551, $p=0.0005$) and reduced the decrease in systemic vascular resistance ($p<0.0017$) and mean arterial pressure ($p<0.005$) responses and the increase in heart rate ($p<0.015$). Atenolol is roughly equipotent to propranolol and was, therefore, used at an equivalent dose as used in previous studies (Branch and Knuepfer, 1992; Knuepfer et al., 1998).

We also examined the role of muscarinic cholinoceptors using atropine methylbromide (0.5 mg/kg), methoctramine (0.3 mg/kg) and pirenzipine (0.02 mg/kg). This dose of atropine attenuated responses to acetylcholine (Knuepfer et al., 1989). The doses of pirenzipine and methoctramine as M_1- and M_2-muscarinic receptor antagonists were obtained from previous studies (MacIagan et al., 1989; Wellstein and Pitschner, 1988). These doses were reported to be effective without significant effects on other muscarinic receptors although they may also interfere with M_4-cholinoceptors at these doses (Eglen and Watson, 1996).

Drugs Used and Statistical Analyses:

Ketamine (Ketaset III ®) and sodium pentobarbital (Nembutal) were obtained from Fort Dodge Pharmaceuticals (Fort Dodge, IA). Xylazine (Rompun ®) was obtained from Bayer Corporation, Agricultural Division (Shawnee Mission, KS). Cefazolin (Geneva Pharmaceuticals/ Marsam Pharmaceuticals, Cherry Hill, NJ) was also used. Pirenzipine dihydrochloride, methoctramine tetrahydrochloride, atenolol, propranolol and atropine methyl bromide were obtained from Sigma Chemical Co. (St. Louis, MO). ICI 188,551 was obtained from Research Biochemicals, Inc. (Natick, MA). Cocaine hydrochloride was provided by the National Institute for Drug Abuse.

Statistical analysis of the data was performed by analysis of variance (ANOVA) for data at multiple time points using a paired approach since control values (cocaine after saline administration) were obtained before examining the effects of each selective antagonist. Specifically, data were obtained at the time of the peak increase in arterial pressure and at 1, 3 and 5 minutes after cocaine administration. Post hoc analysis was performed at individual time points using Dunn's (Bonferonni) procedure.

In addition, hemodynamic data were obtained at the time of the maximum decrease in cardiac output (since all rats were vascular responders). The maximum change in cardiac output was typically observed during the first minute after cocaine administration. Responses obtained at the time of the maximum decrease in cardiac output or to the effects of agonists were analyzed separately using a Students' paired t analysis on saline pretreated vs. drug pretreated data from individual rats. The hemodynamic responses at the time of the maximum decrease in cardiac output after propranolol pretreatment were analyzed with one-tailed tests since previous published work from our laboratory demonstrated significant changes in cocaine-induced responses after propranolol pretreatment. All analyses were performed using GBStat (Dynamic Microsystems, Inc., Silver Springs, MD). Significant changes were determined if $p<0.05$.

Results

We examined 18 rats (vascular responders) for their responses to cocaine before and after administration of selective receptor antagonists. The resting hemodynamic values for these rats are shown in Table 1. Cocaine produced an increase in arterial pressure and systemic vascular resistance and a decrease in cardiac output (Figure 1). As previously observed, hindquarters vascular resistance was increased during the initial pressor response then became a vasodilation. Likewise, stroke volume had a biphasic response with an initial decrease followed by an increase (Figure 1). Heart rate had an initial variable response followed by a consistent decrease.

The contribution of the hindquarters response to the overall increase in systemic vascular resistance was examined by correlating the average resistance responses in individual rats. Linear regression analysis (Figure 2) demonstrated a significant correlation ($p=0.0026$) between the extent of hindquarters and systemic vascular resistance changes at the time of the maximum decrease in cardiac output (often coinciding with the peak increase in systemic vascular resistance) suggesting that the skeletal muscle response contributes significantly to the increase in systemic vascular resistance.

Effects of selective β-adrenergic receptor antagonists

Previous work from our laboratory examined the effects of propranolol on hindquarters and cardiac output responses in separate groups of instrumented conscious rats (Branch and Knuepfer, 1992). Therefore, propranolol (1 mg/kg, i.v.) was administered to a small number of rats ($n=5$) instrumented for both cardiac output and hindquarters flow determination to verify previous observations. Propranolol pretreatment decreased heart rate ($t = 7.7$, $df = 4$, $p<0.002$) and increased stroke volume ($t = -3.3$, $df = 4$, $p<0.03$, Table 2) as observed in previous studies

(Branch and Knuepfer, 1992, 1994a). The net result was a slight decrease in cardiac output responsiveness that did not reach significance ($p<0.052$, Table 2).

Propranolol pretreatment altered hemodynamic responses to cocaine at the time of the maximum cardiac output response such that the cardiac output ($t = 2.9$, $df = 4$, $p<0.022$, one-tailed) and stroke volume ($t = 2.7$, $df = 4$, $p<0.028$, one-tailed) responses were more negative and the initial hindquarters vasoconstriction was greater ($t = -2.4$, $df = 4$, $p<0.04$, Figure 3). As previously reported (Branch and Knuepfer, 1992, 1994a; Knuepfer et al., 1998), propranolol enhanced the delayed (1, 3 and 5 min after cocaine) pressor response and increase in systemic vascular resistance apparently by preventing the hindquarters vasodilation and despite a greater reduction in cardiac output (data not shown).

Pretreatment with the β_1-adrenoceptor selective agent, atenolol (1 mg/kg, iv), decreased heart rate ($t = 6.62$, $df = 9$, $p=0.001$) and cardiac output ($t = 5.48$, $df = 9$, $p = 0.0004$) while increasing stroke volume ($t = -3.61$, $df = 9$, $p < 0.006$) and systemic vascular resistance ($t = -5.64$, $df = 9$, $p = 0.0003$, Table 2). Subsequent administration of cocaine resulted in a greater decrease in cardiac output ($F_{1,19}=15.3$, $p=0.001$) and stroke volume ($F_{1,19}=13.34$, $p<0.002$) and a greater increase in systemic vascular resistance ($F_{1,19}=6.56$, $p<0.02$) without significantly affecting hindquarters vascular resistance (Figure 4). At the time of the maximum decrease in cardiac output, only the cardiac output response was significantly enhanced by atenolol pretreatment ($t = 4.06$, $df = 9$, $p < 0.003$, Figure 3).

Pretreatment with the β_2-adrenoceptor selective antagonist, ICI 118,551 (0.5 mg/kg, iv), caused an increase in hindquarters vascular resistance ($t = -3.04$, $df = 11$, $p<0.02$) and decreases in cardiac output ($t = 3.18$, $df = 11$, $p<0.009$) and heart rate ($t = 2.92$, $df = 11$, $p<0.014$, Table 2). Administration of cocaine, 10 min after ICI 118,551, resulted in an enhancement of the pressor

response ($F_{1,23}$=40.9, p<0.0001) due to a greater increase in systemic vascular resistance ($F_{1,22}$=15, p<0.001) and despite a more negative cardiac output response ($F_{1,23}$=7.3, p=0.013, Figure 5). This appeared to result, at least in part, to prevention of the hindquarters vasodilation ($F_{1,22}$=12.3, p=0.0021, Figure 5).

The maximum decrease in cardiac output was not significantly altered by ICI 118,551 (p = 0.0501). The decrease in stroke volume and increases in arterial pressure (t = -3.45, df = 10, p = 0.0062) and systemic vascular resistance (t = -4.14, df = 9, p = 0.0025) were enhanced at the time of the maximum decrease in cardiac output (Figure 3).

Effects of selective muscarinic receptor antagonists

Atropine methylbromide (0.5 mg/kg, i.v.) was administered to 5 rats to verify results from previous studies studying the effects of cocaine on cardiac output and hindquarters vascular resistance in separate groups of animals (Knuepfer and Branch, 1992; Knuepfer et al., 1999). As previously noted, atropine administration alone elicited an increase in heart rate (t = -6.1, df = 4, p<0.004, Table 2). Atropine pretreatment attenuated the decrease in cardiac output (t = -4.7, df = 4, p<0.005) but did not affect hindquarters vascular resistance at the time of the maximum decrease in cardiac output (Figure 3).

Administration of the M_1 selective muscarinic antagonist, pirenzipine (0.02 mg/kg) did not alter hemodynamic values significantly (Table 2). Pirenzipine pretreatment did not alter the time course of hemodynamic responses to cocaine significantly (Figure 6). In contrast, at the time of the peak decrease in cardiac output, the arterial pressure response (t = 2.51, df = 8, p<0.041) and the increase in systemic vascular resistance (t = 2.51, df = 9, p = 0.033) were reduced (Figure 3). The decrease in cardiac output responsiveness was not significant (p = 0.068).

Pretreatment with the M_2 antagonist, methoctramine (0.3 mg/kg, i.v.), resulted in a significant increase in arterial pressure ($t = -2.81$, $df = 8$, $p<0.023$). This was due, in part, to a substantial increase in heart rate ($t = -8.79$, $df = 8$, $p<0.0001$) although the decrease in stroke volume ($t = 4.31$, $df = 8$, $p<0.0027$, Table 2) counteracted the pressor response. Methoctramine pretreatment reduced the pressor response ($F_{1,17}=12.2$, $p=0.003$) to cocaine by reducing the increase in systemic vascular resistance ($F_{1,17}=7.6$, $p<0.014$) without affecting hindquarters vascular resistance (Figure 7). Methoctramine pretreatment also attenuated the decrease in cardiac output ($F_{1,17}=4.8$, $p<0.043$, Figure 7) observed in vascular responders. The increase in the pressor ($t = 2.85$, $df = 8$, $p<0.022$) and systemic vascular resistance responses ($t = 4.03$, $df = 8$, $p<0.004$) and the decrease in cardiac output ($t = -2.8$, $df = 8$, $p<0.024$) were significantly attenuated at the time of the peak decrease in cardiac output (Figure 3).

Discussion

The present results suggest that the cardiac responses to cocaine are modulated by β_1-adrenoceptors and M_2 cholinoceptors whereas β_2-adrenoceptors in the hindquarters vasculature attenuate the increase in systemic vascular resistance in rats with a large increase in systemic vascular resistance in response to cocaine (vascular responders). These findings better characterize the mechanisms by which cocaine alters arterial pressure and, perhaps, the compensatory responses such as skeletal muscle vasodilation, that limit the pressor responses to cocaine. Although we have reported similar regional and systemic vascular responses in previous studies (Branch and Knuepfer, 1992, Knuepfer and Branch, 1992, Knuepfer et al., 1994), this is the first study where we recorded both in the same rats. This allowed us to directly compare the magnitude of the hindquarters vascular resistance directly with the systemic vascular resistance and cardiac output responses. We observed a significant relationship between the magnitude of the hindquarter vascular response and the increase in systemic vascular resistance suggesting that skeletal muscle vasodilation is important in moderating the hemodynamic response pattern to cocaine in vascular responders.

We reported that the non-specific β-adrenoceptor antagonist propranolol (1 mg/kg), prevented the hindquarters vasodilation and enhanced the delayed pressor response and increase in systemic vascular resistance to cocaine administration (Branch and Knuepfer, 1992, 1994a; Knuepfer et al., 1998). Likewise, Kenny et al. (1992) reported that propranolol pretreatment enhanced the increase in systemic vascular resistance in conscious dogs although the pressor response to cocaine was attenuated. We have evidence that this response is mediated, at least in part, by central β-adrenoceptors since intracerebroventricular administration of propranolol alters hemodynamic responses to cocaine in a similar manner as intravenous propranolol (Dong et al.,

2001). Both ICI 118,551 and, to a lesser extent, atenolol, enhanced the decrease in cardiac output and increase in systemic vascular resistance but only the β_2-antagonist prevented the hindquarters vasodilation. This suggests a role for β_2-adrenoceptors in vasodilation. Kirby and Johnson (1990) demonstrated a dependence of the hindquarters vasodilator response to acute stress on β_2-adrenoceptors. Since we have noted many similarities in the hemodynamic responses to acute stress, particularly startle, and to cocaine (Knuepfer et al., 2001), it is not surprising that similar autonomic mechanisms are involved.

Previous studies suggest that the hindquarters and systemic vascular resistance responses are dependent on adrenal catecholamine release (Branch and Knuepfer, 1994a; Knuepfer and Branch, 1992). We reported that cocaine elicits hindquarters vasodilation even under anesthesia suggesting that it is not use dependent (Knuepfer and Branch, 1992). The response also depends on prostaglandins since the delayed hindlimb vasodilation to cocaine is reversed by ibuprofen or BW755C pretreatment (Knuepfer et al., 1994). Evidence suggests that cocaine acts by increasing sympathoadrenal activity in the CNS in humans (Jacobsen et al., 1997; Vongpatanasin et al., 1999) and animals (Abrahams, Cuntapay and Varner, 1996; Branch and Knuepfer, 1994b; Chiueh and Kopin, 1978; Kiritsy-Roy et al., 1990; Purcell et al., 2000). Therefore, we propose that centrally-acting sympathomimetics like cocaine elicit hindquarters vasodilatory responses.

An alternative explanation for the effects of propranolol on hindquarters vasodilation and lack of response to atenolol may be due to differences in the lipophilicity of these agents. Propranolol crosses the blood-brain barrier without difficulty whereas atenolol does not get into the CNS readily (McAinsh and Cruickshank, 1990; Van Zwieten and Timmermanns, 1979). This is not likely to explain the actions of propranolol since we did not observe any differences in systemic vascular resistance responses to acute cold stress exposure after

intracerebroventricular metoprolol (30 μg) administration (Rauls, Tan and Knuepfer, unpublished observations). We reported that the initial effects of cold water exposure (startle response) elicits similar hemodynamic responses as cocaine and is mediated by pharmacologically similar mechanisms (Knuepfer et al., 2001). This evidence provides further support for the role of peripheral β_2-adrenoceptors in ameliorating the vascular responses to cocaine.

In earlier studies, we reported that pretreatment with atropine methyl bromide (0.5 mg/kg, i.v.) reduced the decrease in cardiac output evoked by cocaine administration in vascular responders and enhanced the hindquarters vasodilation (Knuepfer and Branch, 1992; Knuepfer et al., 1999). In the present study, we noted a significant attenuation of the decrease in cardiac output after atropine or methoctramine pretreatment but not after pirenzipine suggesting that the cardiodepression in vascular responders is mediated by M_2 muscarinic receptors in the myocardium. Although we did not note significant changes in the hindquarters response to cocaine, there was a significant reduction in the increase in systemic vascular resistance that was responsible for the attenuation of the pressor response (Figure 3). Interestingly, methoctramine had a profound effect in reducing the pressor response and increase in systemic vascular resistance that was not revealed by pretreatment with atropine (Knuepfer and Branch, 1992; Knuepfer et al., 1999). The effect on systemic vascular resistance may result from blockade of peripheral M_2-cholinoceptors of vagally mediated catecholamine release from sympathetic nerve terminals as suggested by Shannon et al. (1993) in studies on the coronary vasculature of conscious dogs. In rats, cocaine elicits a bradycardia that is attenuated by atropine pretreatment suggesting an increase in vagal tone (Knuepfer and Branch, 1992; Knuepfer et al., 1999). Our data are consistent with this hypothesis. In contrast, others reported that muscarinic receptors

inhibit catecholamine release from sympathetic nerves (Lavallée et al., 1978; Muscholl, 1980) due to activation of M_2-cholinoceptors (Yokitani and Osumi, 1993). This would be expected to produce the opposite effects as those seen in our study. Alternatively, methoctramine may block presynaptic receptors that inhibit nitric oxide release thereby reversing the increase in systemic vascular resistance. Although it is not known whether this occurs widely in the vasculature, this has been demonstrated in porcine cerebral blood vessels (Liu and Lee, 1999).

The effects of methoctramine may also be due to blockade of a direct cholinergic effect of cocaine. Some have suggested that cocaine inhibits muscarinic receptor binding (Flynn et al., 1992). In contrast, Sharkey et al. (1988) reported that (-)-cocaine inhibits M_2 muscarinic cholinergic receptor binding in the heart and brain with a K_i of 18 μM. This concentration (equivalent to 5-6 μg/ml) may exceed that noted in humans (Foltin et al., 1995; Javaid et al., 1978) or animals (Branch and Knuepfer, 1994b; Nayak et al., 1976) except after exposure to very high levels as described previously (Knuepfer, 2003). Therefore, the contribution of direct binding of cocaine to muscarinic receptors may not be relevant in most experimental or clinical cases.

We have additional evidence for cholinergic involvement in cocaine responses. The non-selective cholinesterase inhibitor, physostigmine (0.1-0.2 mg/kg) or neostigmine (0.1 mg/kg) reduced the increase in arterial pressure and systemic vascular resistance elicited by cocaine without altering the cardiac output response. Likewise, neither the selective butyrylcholinesterase inhibitor, tetraisopropyl pyrophosphamide (iso-OMPA, 0.5 mg/kg) nor enhancing cholinesterase activity with human butyrylcholinesterase (9.9 mg/kg) altered the cardiac output responses (Knuepfer et al., 1997). Therefore, we concluded that the toxic effects of higher doses of physostigmine were mediated by parasympathetic cholinergic nerves rather

than by reducing cocaine metabolism. This suggested that the effects noted with atropine are a result of blocking activation of muscarinic receptors not a direct cholinergic effect of cocaine since physostigmine did not alter the cardiac output response (Knuepfer and Gan, 1999).

It could be argued that the lack of effects of the M_1 antagonist, pirenzipine, is due to an inadequate dosage for M_1-cholinoceptor blockade. A dose of 1.1 mg was used in humans to block M_1 receptors (Wellstein and Pitschner, 1988). Assuming a 70 kg volume of distribution, this dose of pirenzipine (0.016 mg/kg) is reported to be more than 3-4 fold of the K_i dose of pirenzipine in an M_1-cholinoceptor assay of plasma samples (Wellstein and Pitschner, 1988). The dose used in our experiment is equivalent to 47 nmol/kg, a dose that has been shown to be selective for the M_1-muscarinic receptors on sympathetic nerves without significantly affecting the M_2 receptors that inhibit neurotransmission (MacIagan et al., 1989). Higher doses (> 100 nmol/kg) also block M_2- and M_3-cholinoceptors making the drug less specific and often eliciting opposite functional effects (MacIagan et al., 1989). Both methoctramine and pirenzipine have high affinity for M_4-cholinoceptors and low affinity towards M_3-cholinoceptors further complicating the interpretation of their effects on cocaine responses (Eglen and Watson, 1996). Nonetheless, pirenzipine binds with higher affinity to excitatory receptors on sympathetic nerves whereas methoctramine has higher affinity for cardiac and smooth muscle muscarinic receptors and inhibits adenylyl cyclase and enhances K^+ conductance (Eglen and Watson, 1996; Hammer and Giachetti, 1982).

In summary, our results suggest that skeletal muscle vasodilation ameliorates the effects of cocaine on arterial pressure and systemic vascular resistance in vascular responders. In contrast, the cardiac responses to cocaine were attenuated by M_2 receptor blockade and exacerbated by β_1-blockade and, to a lesser extent, by β_2-blockade. Therefore, the hemodynamic

responses to cocaine are dependent on activation of specific adrenergic and cholinergic receptors.

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Footnote

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Figure Legends

Figure 1: Time course of the hemodynamic responses to cocaine (5 mg/kg, i.v.) in all rats (n=18). The mean arterial pressure (MAP), systemic vascular resistance (SVR), hindquarters vascular resistance (HqR), cardiac output (CO), heart rate (HR) and stroke volume (SV) are depicted for 5 minutes after the initial pressor response.

Figure 2: Correlation between the average change in hindquarters vascular resistance (HqR) and the systemic vascular resistance (SVR) at the time of the maximum change in cardiac output. The linear relationship was significant (R=0.64, p=0.0026).

Figure 3: Hemodynamic responses to cocaine (5 mg/kg, i.v.) at the time of the maximum change in cardiac output after pretreatment with saline (solid bars) and after pretreatment with selective receptor antagonists for the β-adrenoceptors (B), β_1-adrenoceptors (B1), β_2-adrenoceptors (B2), muscarinic cholinergic receptors (M), M_1 muscarinic receptors (M1) and M_2 muscarinic receptors (M2). Other abbreviations are described in Fig. 1. Significant effects of drug treatment were determined using a paired Students' t test and are denoted with an asterisk)

Figure 4: Time course of the hemodynamic responses to cocaine (5 mg/kg, i.v.) before and after pretreatment with atenolol (1 mg/kg, i.v.). The initial time point demonstrates the change from baseline due to the drug pretreatment alone. Data were analyzed with a two-way analysis of variance. Significant drug effects ($p<0.05$) are denoted with an asterisk. Abbreviations are described in Fig. 1.

Figure 5: Time course of the hemodynamic responses to cocaine (5 mg/kg, i.v.) before and after pretreatment with ICI 118,551 (0.5 mg/kg, i.v.). Data were analyzed with a two-way analysis of variance. Significant drug effects ($p<0.05$) are denoted with an asterisk. Abbreviations are described in Fig. 1.

Figure 6: Time course of the hemodynamic responses to cocaine (5 mg/kg, i.v.) before and after pretreatment with pirenzipine (0.02 mg/kg, i.v.). Data were analyzed with a two-way analysis of variance. Significant drug effects ($p<0.05$) are denoted with an asterisk. Abbreviations are described in Fig. 1.

Figure 7: Time course of the hemodynamic responses to cocaine (5 mg/kg, i.v.) before and after pretreatment with methoctramine (methoctr., 0.3 mg/kg, i.v.). Data were analyzed with a two-way analysis of variance. Significant drug effects ($p<0.05$) are denoted with an asterisk. Abbreviations are described in Fig. 1.

Table 1: Control values

Drug	N	MAP (mmHg)	Heart Rate (b/min)	Cardiac Output (kHz shift)	Hindquarters Flow (kHz shift)
Propranolol	5	110 ± 4	388 ± 8	8.3 ± 1.1	8.1 ± 1.6
Atenolol	10	113 ± 4	406 ± 15	10.2 ± 0.5	5.8 ± 0.7
ICI 118,551	12	112 ± 3	399 ± 8	9.8 ± 0.6	8.2 ± 0.8
Atropine MeBr	5	106 ± 4	380 ± 15	9.3 ± 1.4	7.4 ± 1.3
Pirenzipine	10	109 ± 2	421 ± 9	10.2 ± 0.4	5.5 ± 1.0
Methoctramine	10	108 ± 3	403 ± 13	9.3 ± 0.6	7.6 ± 1.0

Table 2: Effects of selective receptor antagonists

Drug	**Change in MAP (mmHg)**	**Change in HR (b/min)**	**Change in CO (%)**	**Change in SV (%)**	**Change in SVR (%)**	**Change in HqR (%)**
Propranolol	-2 ± 3	-52 ± 8**	-5.0 ± 1.7	10 ± 3**	3 ± 2	-3 ± 4
Atenolol	-1 ± 3	-82 ± 13**	-13.2 ± 2.3**	8 ± 2**	16 ± 4**	11 ± 10
ICI 118,551	1 ± 2	-29 ± 8*	-3.8 ± 1.5**	3 ± 3	5 ± 2	20 ± 7*
Atropine MeBr	8 ± 4	73 ± 13**	4.3 ± 6.7	-13 ± 6	5 ± 11	7 ± 18
Pirenzipine	-1 ± 2	-16 ± 10	-0.8 ± 3.9	3 ± 4	2 ± 5	-3 ± 4
Methoctramine	9 ± 3*	88 ± 9**	2.5 ± 2.7	-16 ± 3**	7 ± 4	1 ± 8

Values obtained 10 min post-drug administration

* $p<0.05$, ** $p<0.01$

Figure 1

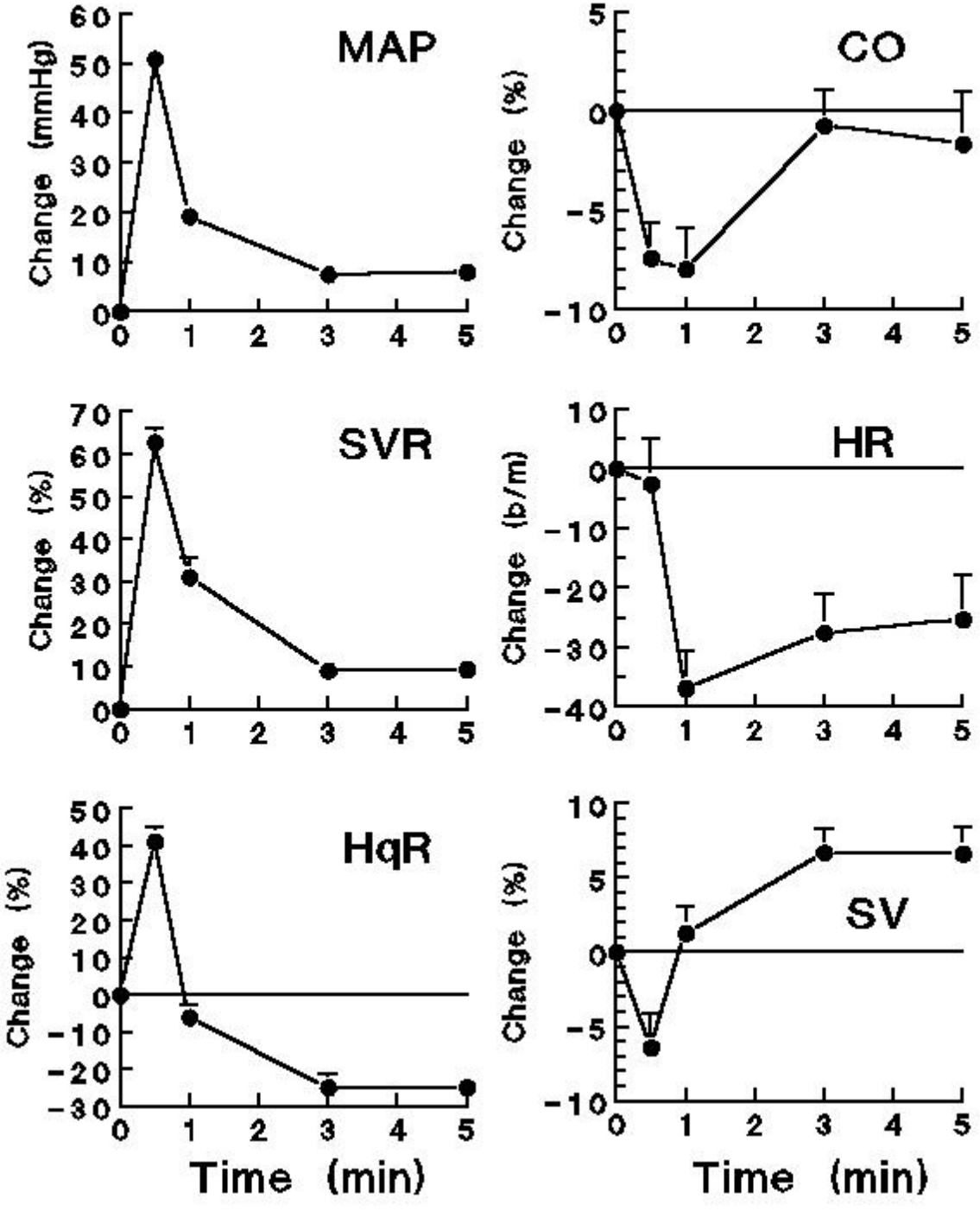

MAP
Change (mmHg)
60
50
40
30
20
10
0
0 1 2 3 4 5
CO
Change (%)
5
0
-5
-10
0 1 2 3 4 5
SVR
Change (%)
70
60
50
40
30
20
10
0
0 1 2 3 4 5
HR
Change (b/m)
10
0
-10
-20
-30
-40
0 1 2 3 4 5
HqR
Change (%)
50
40
30
20
10
0
-10
-20
-30
0 1 2 3 4 5
Time (min)
SV
Change (%)
10
5
0
-5
-10
0 1 2 3 4 5
Time (min)

Figure 2

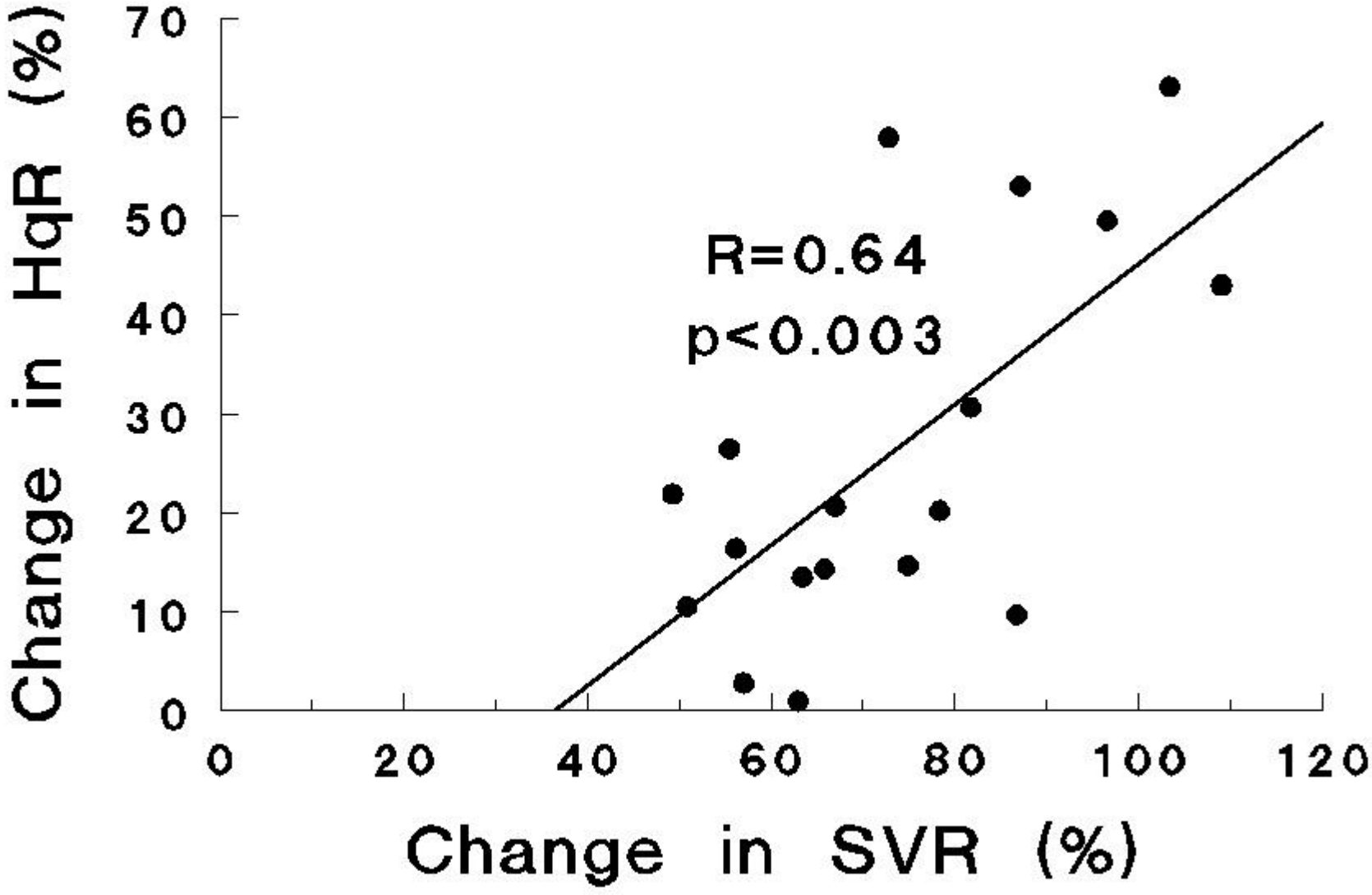

Change in HqR (%)
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
Change in SVR (%)
R=0.64
p<0.003

Figure 3

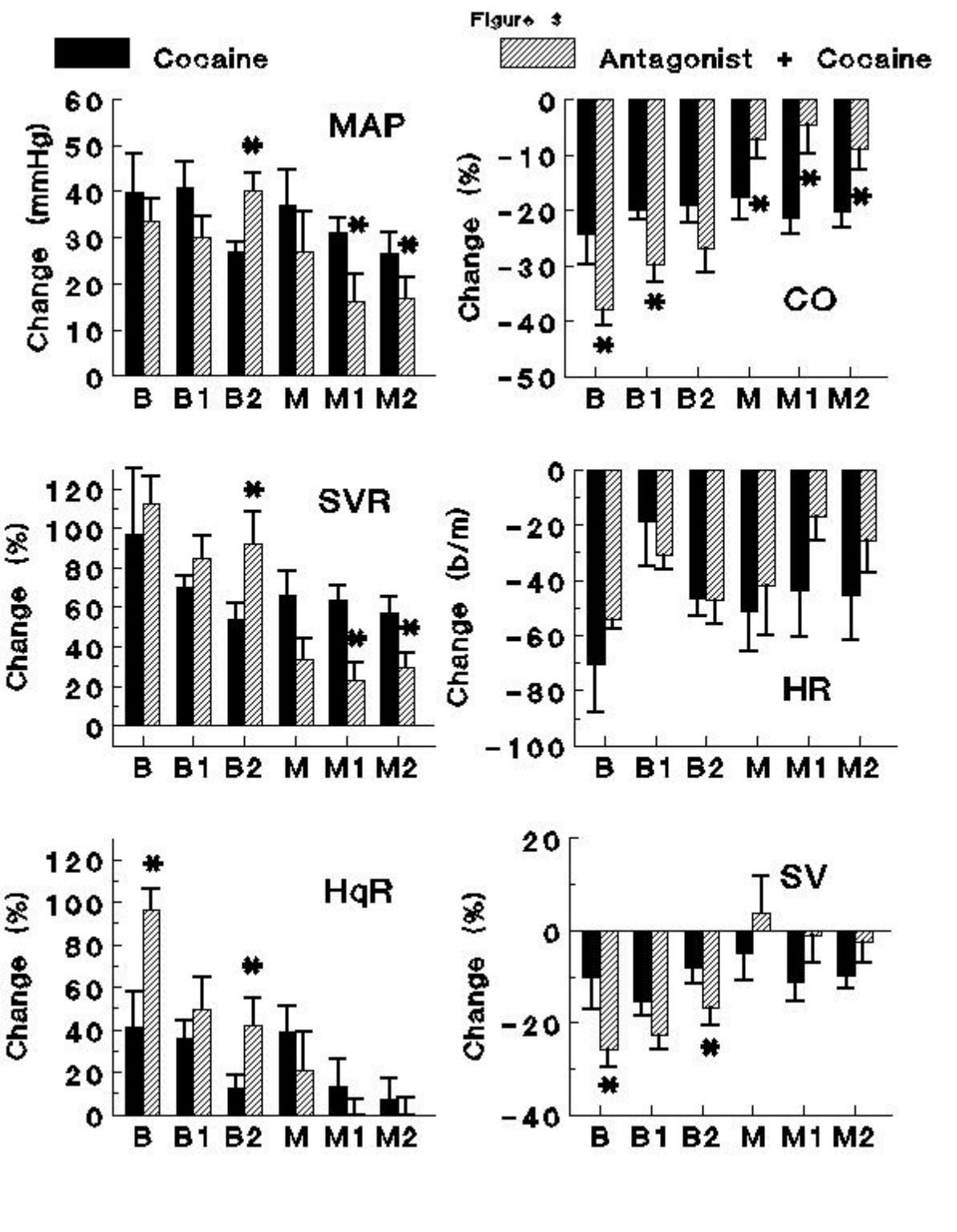

Cocaine
Antagonist + Cocaine
MAP
Change (mmHg)
60
50
40
30
20
10
0
B B1 B2 M M1 M2
CO
Change (%)
0
-10
-20
-30
-40
-50
SVR
Change (%)
120
100
80
60
40
20
0
HR
Change (b/m)
-100
-80
-60
HqR
SV
-40

Figure 4

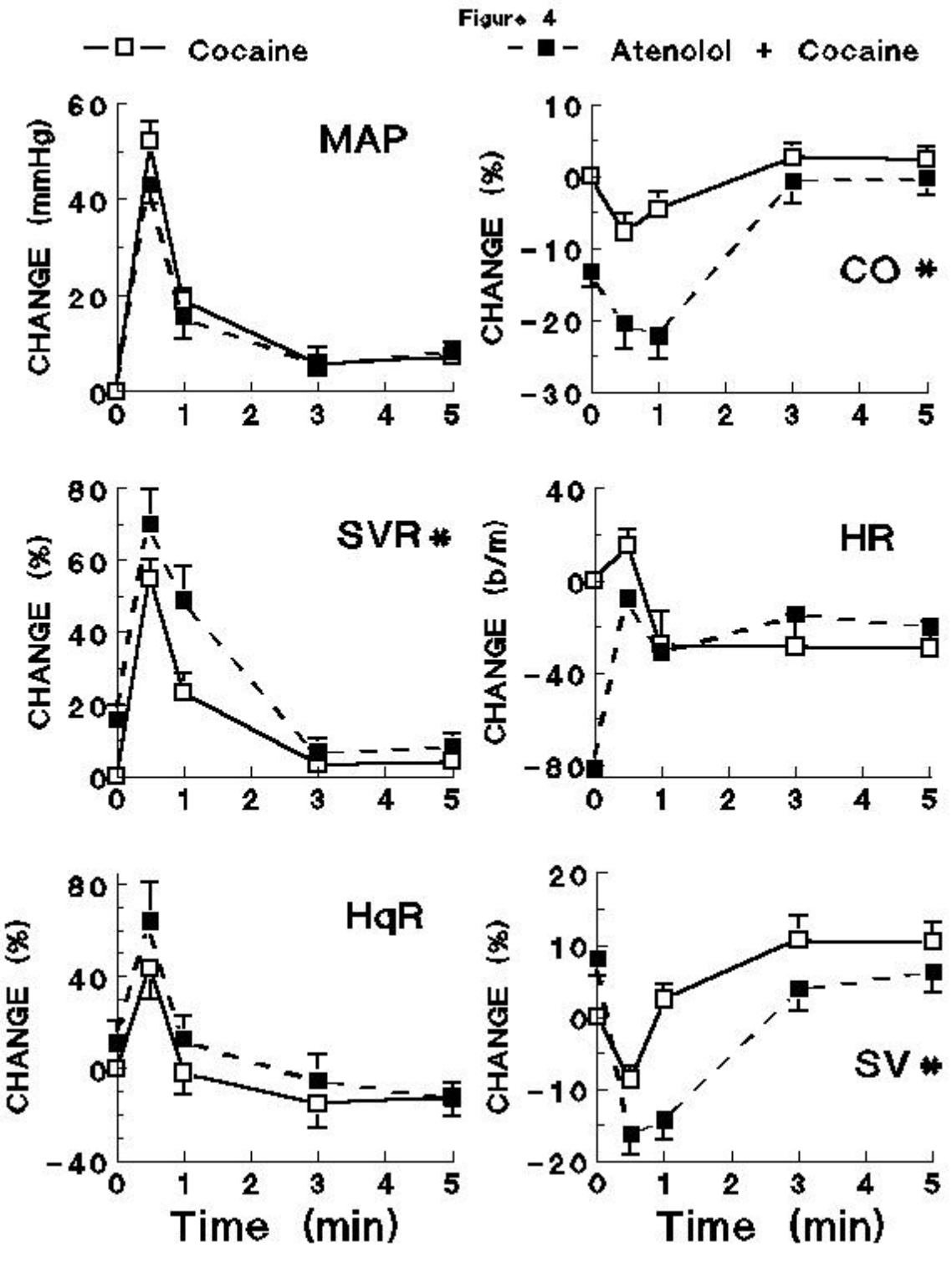

Cocaine
Atenolol + Cocaine
MAP
CO *
SVR *
HR
HqR
SV *
CHANGE (mmHg)
CHANGE (%)
CHANGE (b/m)
Time (min)
Time (min)

Figure 5

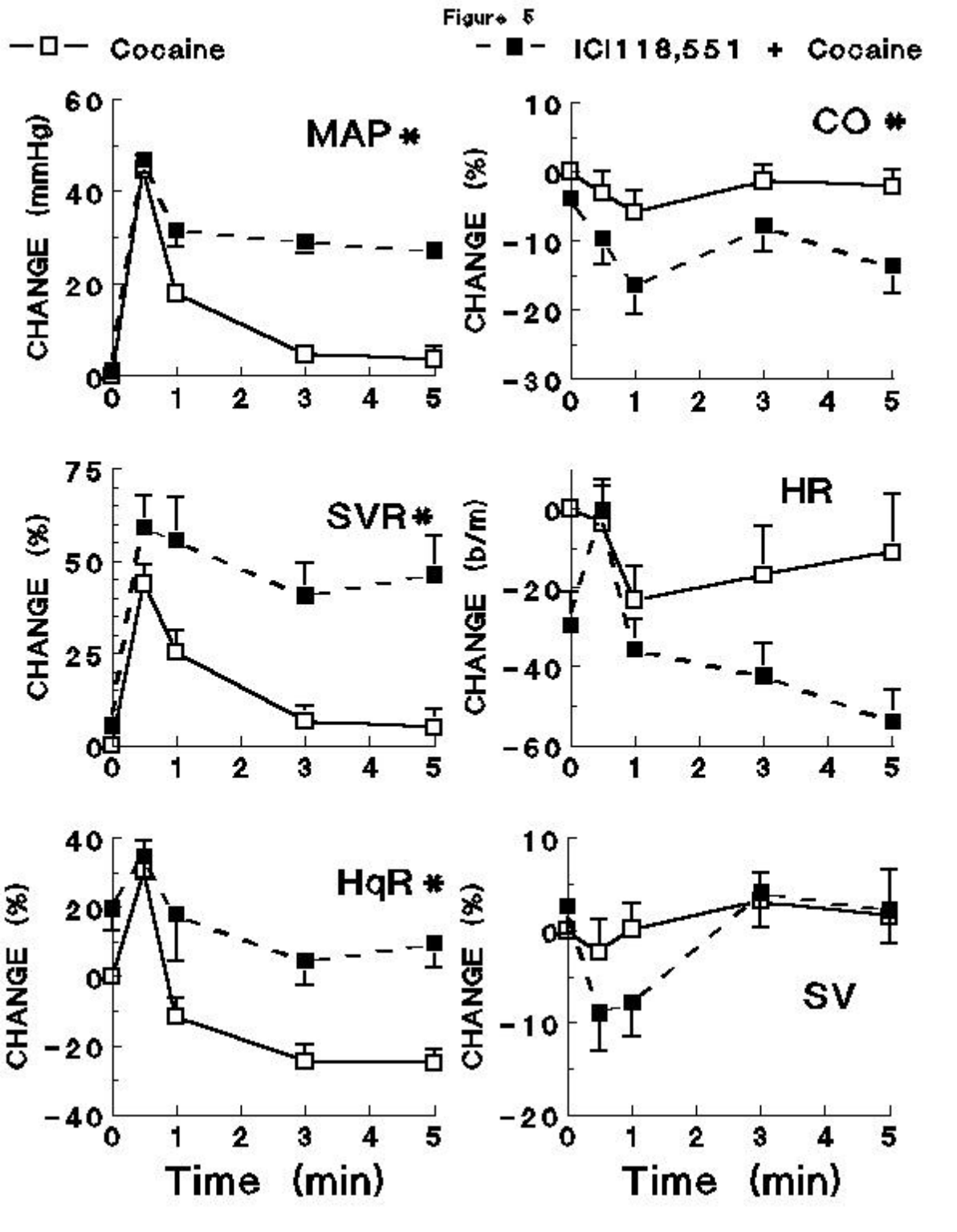

Cocaine
ICI118,551 + Cocaine
MAP *
CO *
SVR *
HR
HqR *
SV
CHANGE (mmHg)
CHANGE (%)
CHANGE (b/m)
Time (min)
Time (min)

Figure 6

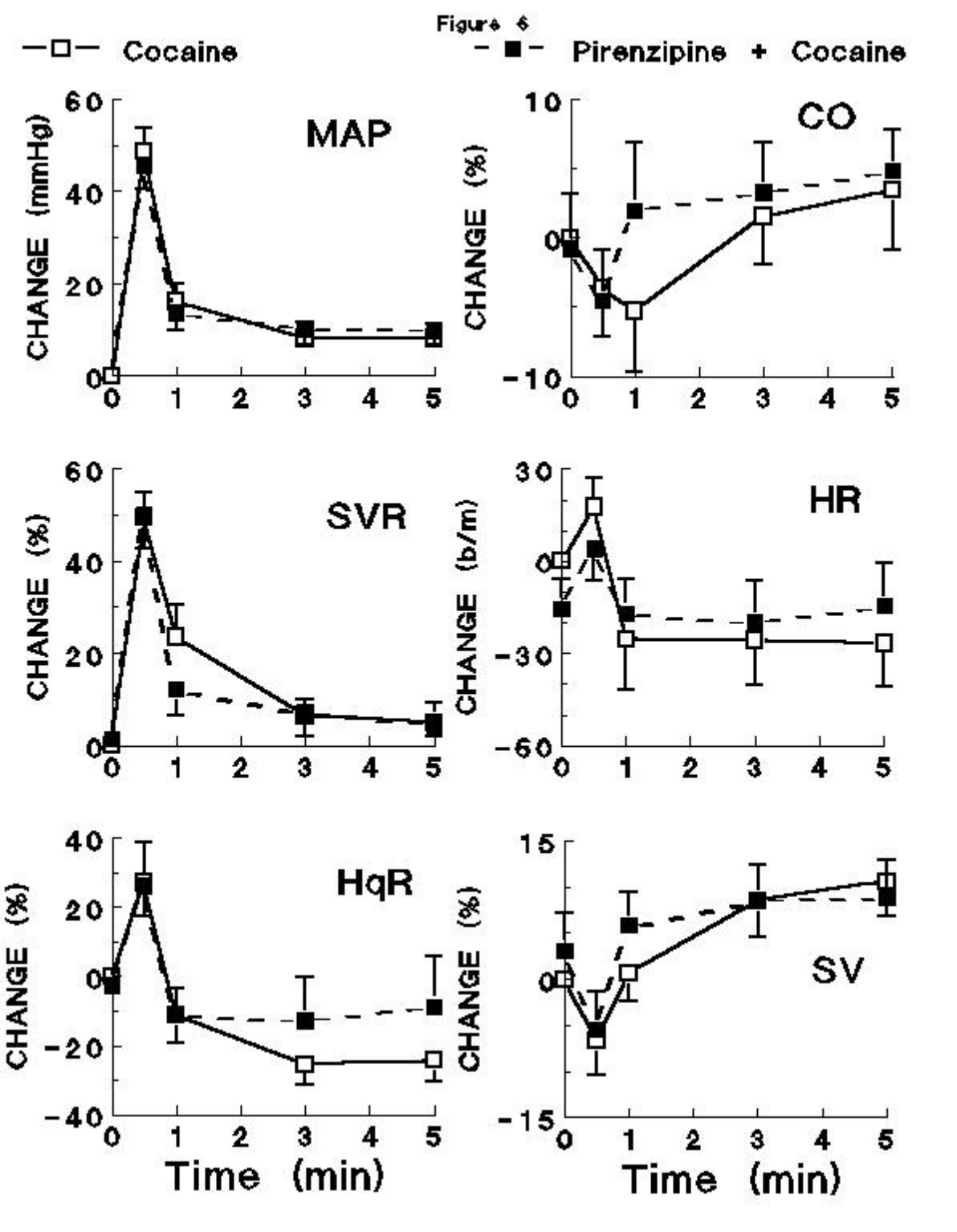
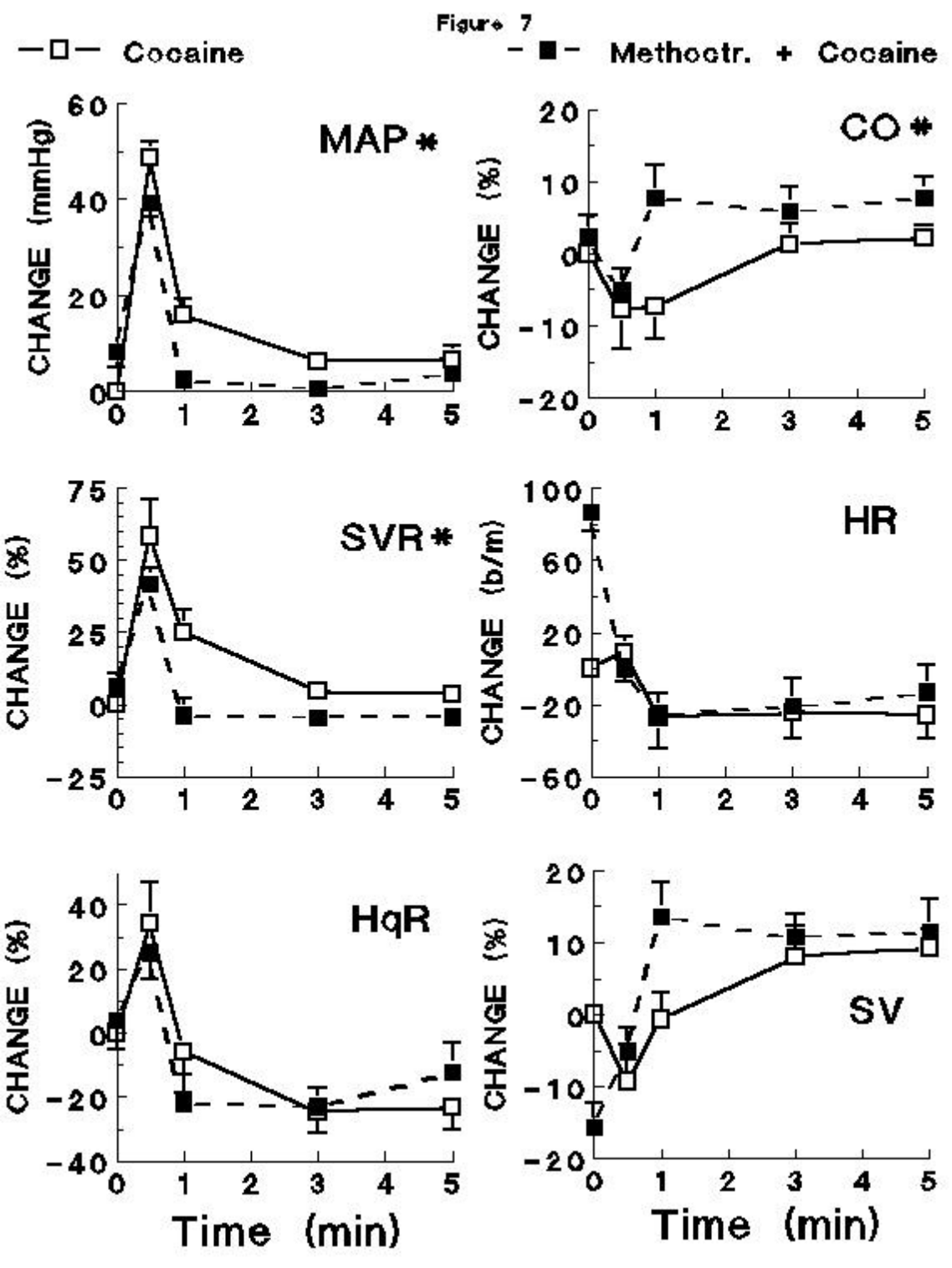

Cocaine
Pirenzipine + Cocaine
MAP
CO
SVR
HR
HqR
SV
CHANGE (mmHg)
CHANGE (%)
CHANGE (b/m)
Time (min)

Figure 7


Cocaine
Methoctr. + Cocaine
MAP *
CO *
SVR *
HR
HqR
SV
CHANGE (mmHg)
CHANGE (%)
CHANGE (b/m)
Time (min)
Time (min)