Diazepam Protects Against the Enhanced Toxicity of Cocaine Adulterated With Atropine

Daniela Braida¹, Alessia Zani¹, Valeria Capurro¹, Giuseppe Rossoni¹, Simona Pegorini¹, Enzo Gori², and Mariaelvina Sala¹,²,*

¹Department of Pharmacology, Chemotherapy and Medical Toxicology, ²Behavioural Pharmacology and Drug Dependence Center, University of Milan, Via Vanvitelli 32, 20129 Milan, Italy

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Abstract. We examined the toxicity of cocatropine (cocaine/atropine mixture) and the therapeutic potential of diazepam on some behavioral and physiological parameters in rats. Atropine (20 and 60 mg/kg) or cocaine (40 mg/kg) alone did not induce any seizure or death, but the combination significantly increased both, after both acute and binge treatment. There was a significant increase of EEG mean total spectral power in cocatropine- in comparison with cocaine-treated animals. Hyperlocomotion was observed in non-seizuring rats treated with cocaine or cocatropine. Cocaine, atropine 60, and cocatropine (40 + 20 and 40 + 60) all induced hyperthermic effects in non-seizuring rats, while cocatropine (40 + 60)-seizuring animals had hypothermia. An initial hypertensive and tachycardiac effect within 15 min was followed by a secondary fall in the cocatropine (40 + 60) group. Cocatropine toxicity was partially or fully reversed by diazepam (5 mg/kg), given intraperitoneally after the first seizure. The present findings provide, for the first time, details of a synergistic toxic effect of the cocaine/atropine mixture and of the potential of diazepam for treating cocatropine-related hospital emergencies.

Keywords: drug abuse, crystalline, anticholinergic, binge, behavior

Introduction

Illicit drugs are frequently adulterated with other chemicals, added either to increase the apparent quantity of the street drug or to enhance its effect. Cocaine is no exception. More than 38 pharmacologically active substances have been reportedly used with cocaine. These include local anesthetics (e.g., procaine, lidocaine, tetracaine), other stimulants (e.g., amphetamine, caffeine, methylphenidate, strychnine), lysergic acid diethylamide (LSD), phenylcyclidine (PCP), heroin, marijuana, and hashish, and phenytoin. These substances are well known for their potential sequelae, and their use with cocaine may acutely increase morbidity and mortality (1 – 3).

At the end of 2004 and the beginning of 2005, a number of cases of intoxication were reported in Belgium, France, Italy, and the Netherlands, involving a cocaine/atropine mixture (cocatropine) known as crystalline. Atropine is a naturally occurring alkaloid of the Atropa belladonna plant, and, if taken in high doses, can prove fatal (4). Once it became clear that the phenomenon was not confined to any one country and that cases might increase, Sedefov (5) reported the emerging drug combination. In these alerts, the agency advises its partners to inform their networks and health authorities on the symptoms of cocaine/atropine intoxication in order to facilitate early diagnosis. Operators of emergency units reported the following symptoms: excitement or restlessness, psychotic symptoms, hallucinations, seizures, reduced consciousness, tachycardia, hypertension, respiratory failure, dry mouth, and severe mydriasis. One fatal case has been reported in Italy. Toxicological analysis of urine samples indicated cocaine and atropine in respectively 60% – 80% and 40% – 20%. Anticholinergic poisoning by adulterated cocaine is not common but there are only few reports of atropine/cocaine intoxication in humans. Quandt et al. (6) reported 15 cases of intoxication with anticholinergic symptoms such as tachycardia, mydriasis,
marked confusion, and bizarre behavior after inhaling cocaine in patients whose urine samples contained atropine, benzocaine, and procaine. Five cases of acute poisoning after sniffing adulterated cocaine presented a serious anticholinergic syndrome which needed physostigmine. Scopolamine was detected in one of these patients (7). Weiner et al. (8), reported a 39-year-old man, presenting classic symptoms of anticholinergic toxicity, who arrived in the emergency department several hours after nasal insufflation of cocaine. In this case urine samples contained cocaine metabolites as well as atropine. The effect of anticholinergic agents administered with cocaine is variable depending on the species used in the study. Atropine enhanced the central respiratory toxicity of cocaine by acting synergistically at caudal chemosensitive areas of the ventrolateral medulla oblongata in urethane-anesthetized, tracheotomized cats (9). In contrast, given systematically by i.p. injection, atropine had no effect on acute cocaine-induced lethality at doses that are effective in preventing parasympathetic effects and lethality of oxotremorine in rats (10). Pretreatment with relatively high doses of atropine partially reduced the pressor and tachycardiac response to intracisternal injection of cocaine in rats (11). Pretreatment with pirenzepine, the muscarinic M1 receptor antagonist, when given at low doses, reduced cocaine-induced lethality in mice, while the non-selective muscarinic antagonist atropine, administered systematically by i.p. injection, had no effect on cocaine-induced lethality, suggesting that neither central nervous system (CNS) nor peripheral M2 receptors have strong influences on cocaine-induced lethality (12, 13).

The present study was designed to look for an antidote to the potential toxicity of cocaine and atropine in rats when given as a combination. Diazepam was chosen on the basis that it protects against cocaine intoxication in rats (14) and humans (15). Among the toxic effects of cocaine on the CNS and peripheral nervous system (16–18), seizures, hyperactivity, hyperthermia, and cardiovascular dysfunction may be important causes of severe intoxication (19). We therefore investigated the effects in rats of cocaine (40 mg/kg) and atropine (20 and 60 mg/kg) on body temperature, motor activity, seizures, and death, when given acutely or repeatedly, alone or as the combination. Electroencephalographic (EEG) mean total spectral power and cardiovascular parameters were monitored for 2 h after acute treatment. The doses of the two compounds were based on the proportion found in cases of human intoxication (67% and 33%, respectively, for cocaine and atropine).

Materials and Methods

Subjects

Male Wistar rats from Charles River (Calco, Italy), weighing 150 – 175 g on arrival, were used. Animals were housed in individual cages in an air-conditioned colony room with a 12-h light / 12-h dark cycle (lights on at 8:00 h). Food and water were continuously available. The rats were allowed to acclimatize themselves to the environment for a week before surgical implantation of cortical electrodes. Separate groups of animals (ten per group) were used for each test, and each rat was used only once. All the experimental procedures followed the guidelines of the Italian Council on Animal Care, approved by Italian Government Decree No. 33/2004-A. All efforts were made to minimize the number of animals used and their suffering.

Seizures and death

During the first 60 min after treatment, behavioral seizures were assessed on a six-point rating scale according to Moiseev et al. (20): 0, no signs of convolution; 1, shaking the head or twitching individual trunk muscles; 2, repeated clonic spasms of the trunk; 3, clonic spasms of the forelimb(s); 4, tonic-clonic convulsions with the animal falling on its side followed by post-ictal depression; 5, repeated severe tonic-clonic or lethal convulsions. The incidence of seizures and death (%) and the latency to the first seizure (s) were recorded.

Spontaneous motor activity

Motor measurements were taken in a separate group of animals. Spontaneous motor activity was evaluated as previously described (21) in an activity cage with the following dimensions: 43-cm-long × 43-cm-wide × 32-cm-high (Ugo Basile, Varese, Italy), placed in a sound-attenuating room. The cage was fitted with two parallel horizontal infrared beams 2-cm off the floor. Cumulative horizontal movements were counted every 10 min for 60 min, starting 5 min after treatment.

EEG

Surgical procedure: Under anesthesia (450 mg/kg, body weight of chloral hydrate, i.p.; Sigma-Aldrich, MO, St. Louis, USA), all the rats were placed in a stereotaxic instrument and four silver–silver chloride ball electrodes were fixed epidurally with dental acrylic cement, as described in detail elsewhere (22), on the right and left of the parieto-occipital cortex according to the Paxinos and Watson brain atlas (23), 2-mm anterior, 2-mm lateral from the midline, and 3-mm posterior from the bregma. The four electrodes, and a fifth inserted into the nasal bone and used as ground, were connected to a
microconnector attached to the rat’s head with dental cement. One week was allowed for recovery after implantation of the electrodes before the experiments were started.

**EEG recording:** The rats were allowed to acclimatize themselves to a sound-attenuated Faraday chamber for a week after surgery. For EEG recording, the microconnector on the rat’s head was connected to a rotating connector attached to the cage and connected to a PC for data acquisition. A PowerLab system (AD Instruments, Castle Hill, Australia) was used for data acquisition and signal processing. Signals were amplified by an Animal Bio-Amplifier (AD Instruments). Spectral powers between 0 and 25 Hz were recorded at a resolution of 0.2 Hz. The recordings were made for 120 min and processed with fast Fourier transform spectral analysis using PC software (PowerLab, AD Instruments Pty Ltd., Australia). The mean spectral power was calculated every 5 min.

**Body temperature**

The experiments were done in the sound-attenuated Faraday chamber. Rats were trained for the temperature measurements for five days before thermoregulator reactions were tested, as described by Sala et al. (24). Briefly, after 1-h acclimatization in the test room, at 22°C, body temperature was measured by inserting a lubricated thermistor probe (PRA-22002-A) (external diameter 3 mm) 2.8 cm into the rectum. The probe was linked to a digital device (CTD 85-M Thermometer; Ellab, Roedovre, Denmark) that displayed the temperature at the tip of the probe with 0.1°C precision. During temperature measurements, made at 10, 30, and 60 min after the last treatment, animals were unrestrained and were held gently by hand at the base of the tail.

**Cardiovascular responses**

Fasted rats were anesthetized with 50 mg/kg, i.p. thiopental sodium salt (Pentothal®; Abbott, Cam- poverde, Latina, Italy). A PE-60 cannula was then placed in the carotid artery on one side and connected to a pressure transducer (HP-1280; Hewlett-Packard, Waltham, MA, USA). A second PE-50 cannula for drug administration was introduced into the left jugular vein. Heart rate (HR) was measured from the blood pressure (MAP) tracing, at the same time. Core body temperature was maintained within narrow limits by a temperature-controlled (37°C) heating-table. After surgery, animals were allowed 20 min to stabilize before drug administration, and MAP and HR were measured continuously for 120 min (PowerLab/400; AD Instruments, Hastings, UK).

**Treatment**

Animals were divided into groups of ten and received the following: saline + saline, saline + cocaine (40 mg/kg), atropine (20 mg/kg) + saline, atropine (60 mg/kg) + saline, saline + diazepam (5 mg/kg), cocatropine (cocaine 40 + atropine 20) + saline, cocatropine (cocaine 40 + atropine 60) + saline, or cocatropine (cocaine 40 + atropine 60) + diazepam. In the last two groups, the second injection of saline or diazepam was given only to rats exhibiting convulsions (at the onset of the first convulsion, about 10 min after cocaine 40 + 60). The remaining rats without convulsions did not receive the second injection. The following drugs were each dissolved in saline and each administered in a volume of 5 ml/kg: cocaine (MacFarlan Smith, Ltd., UK), atropine (Merck, Darmstadt, Germany), and diazepam (Roche, Milan, Italy).

**Seizure and death after repeated administration**

Binge treatment was carried out according to Pradhan et al. (25) and consisted of three injections of cocaine (40 mg/kg) or cocatropine (40 + 20 and 40 + 60 mg/kg) 3-h apart on each of three consecutive days. After a four-day recovery period, the animals again received cocaine or atropine according to the same schedule. After each single treatment, seizures and death were recorded for 60 min.

**Statistics**

The percentages of animals developing seizures or death were analyzed by Fisher’s exact probability test. Mean (±S.E.M.) seizure scores and time to the first seizure were analyzed by Student’s t-test. The other parameters were analyzed by one- or two-way ANOVA followed by Tukey’s or Bonferroni’s test. EEG data of animals showing seizures were analyzed separately. Only temperatures at 60 min (peak effect) were statistically analyzed. Data about seizures and death, evaluated after repeated treatment, were fitted by a sigmoid dose–response curve. The number of treatments needed to reach 50% of seizures or death (TD₅₀) was calculated and compared using Student’s t-test. The level of significance was P<0.05. All statistical analyses were done by using Prism, version 4 software (GraphPad, La Jolla, CA, USA).

**Results**

**Seizures and death**

Table 1 shows the effects of cocaine, atropine, cocatropine, and diazepam on the incidence of seizures and lethality in rats. Atropine (20 and 60) or cocaine (40) alone did not induce any seizure or death, but the
Protection Against Cocatropine Toxicity

Combination at both dosages of atropine led to a significantly higher incidence of seizures. Behavioral seizures started with tonic-clonic activity followed immediately by respiratory arrest and death of 20% – 30% of animals given cocatropine at both dosages. Diazepam completely blocked the seizures and death after cocatropine (40 + 60). In cocatropine-treated groups, there was no difference in the mean time to the first seizure and the mean seizure score at either dosage (Fig. 1).

Spontaneous motor activity
Treatment affected spontaneous motor activity in terms of mean horizontal counts ($F_{9,90} = 9.56, P<0.0001$) (Fig. 2). Post-hoc test indicated that cocaine (40 mg/kg), atropine (20 and 60 mg/kg), and cocatropine (40 + 20 and 40 + 60) significantly increased mean horizontal counts in non-seizuring animals compared to the remaining groups. Diazepam did not affect the spontaneous motor activity in cocatropine (40 + 60)-treated animals showing seizures. However, this last group showed an increased mean number of horizontal counts in comparison to the vehicle and diazepam groups.

EEG
Qualitative representative EEG recordings after different treatments are shown in Fig. 3 (left). Cocaine gave a pattern similar to vehicle, while atropine increased the cortical amplitude. Burst seizure occurred in one rat given cocatropine (40 + 60). Diazepam achieved a partial but robust recovery of normal electrical activity.

The mean total spectral power of cocatropine at peak effect (10 min) is represented in Fig. 3 (right) where one-way ANOVA evidenced a treatment effect ($F_{9,90} = 250.6, P<0.0001$). Atropine and cocaine treatment did not change mean total spectral power. The combination of cocaine and atropine (40 + 60) increased the mean total spectral power in seizuring and not-seizuring animals while the combination of 40 + 20 induced an increase of this parameter only in seizuring animals. Diazepam, which per se was ineffective, partially antagonized the cocatropine (40 + 60)-induced mean total spectral power increase.

Body temperature
The peak temperature change, which was reached at

### Table 1. Effects of cocaine, atropine, cocatropine, and diazepam on seizures and lethality in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Seizures (%)</th>
<th>Lethality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>—</td>
<td>0 (0/15)</td>
<td>0 (0/15)</td>
</tr>
<tr>
<td>Cocaine</td>
<td>40</td>
<td>0 (0/15)</td>
<td>0 (0/15)</td>
</tr>
<tr>
<td>Atropine 20</td>
<td>0 (0/15)</td>
<td>0 (0/15)</td>
<td></td>
</tr>
<tr>
<td>Atropine 60</td>
<td>0 (0/15)</td>
<td>0 (0/15)</td>
<td></td>
</tr>
<tr>
<td>Cocatropine 40 + 20</td>
<td>50** (6/12)</td>
<td>33* (4/12)</td>
<td></td>
</tr>
<tr>
<td>Cocatropine (without seizures)</td>
<td>40 + 60</td>
<td>—</td>
<td>0 (0/6)</td>
</tr>
<tr>
<td>Cocatropine + Saline*</td>
<td>40 + 60</td>
<td>100*** (5/5)</td>
<td>60** (3/5)</td>
</tr>
<tr>
<td>Cocatropine + Diazepam*</td>
<td>40 + 60</td>
<td>0* (0/5)</td>
<td>0 (0/5)</td>
</tr>
</tbody>
</table>

*aSaline or diazepam was given only to animals showing seizures at the time of the first seizure; thus “0” seizures indicates the disappearance of seizures. *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs the corresponding vehicle-injected group; *$P<0.05$ vs cocatropine 40 + 60 + saline (Fisher exact probability test).
60 min after the last treatment, is shown in Fig. 4. ANOVA indicated a significant treatment effect \( (F_{9,90} = 12.19, P < 0.0001) \). Post-hoc test evidenced a significant increase of body temperature in non-seizuring rats after cocaine, atropine 60, cocatropine (40 + 20), and cocatropine (40 + 60). In contrast, there was a hypothermic effect in cocatropine (40 + 60)-seizuring animals. Diazepam, which per se did not affect this parameter, partially, but not significantly, antagonized the cocaine-induced hypothermia.

**Cardiovascular responses**

The cardiovascular responses to cocaine or cocatropine (40 + 60) in anesthetized rats is shown in Fig. 5. The cocaine, cocatropine, and cocatropine + diazepam groups presented a primary peak pressor response within 15 – 20 min (top panel) and tachycardia at 10 min (bottom panel). This was followed by a drop in MAP (to below baseline) in the cocatropine- and cocatropine + diazepam-treated groups, somewhat less marked in the latter. Mean HR also decreased in all except the diazepam and vehicle groups. The cardiovascular responses were normal again by 120 min. Two-way ANOVA for MAP at peak effect indicated significant treatment \( (F_{1,108} = 24.93, P < 0.0001) \), time \( (F_{5,108} = 8.99, P < 0.0001) \), and interaction \( (F_{5,108} = 34.88, P < 0.0001) \) effects (Table 2). Post-hoc comparison showed that cocaine and cocatropine raised MAP in comparison with the other groups at 15 min, while only cocatropine caused a significant decrease at 50 min. Diazepam per se
did not affect cardiovascular parameters, but significantly reversed all the changes induced by cocatropine.

Two-way ANOVA for HR at peak effect indicated significant treatment ($F_{1,108} = 26.87, P < 0.0001$), time ($F_{5,108} = 5.79, P < 0.05$), and interaction ($F_{5,108} = 21.89, P < 0.0001$) effects. Post-hoc comparison showed that cocatropine raised HR at 10 min in comparison with the other groups, with decreases at 35 min in the atropine, cocaine, and cocatropine groups, most marked in the latter. Diazepam reversed this decrease at 50 min.

**Seizure and death after repeated administration**

Repeated doses of cocaine or cocatropine induced behavioral seizures consisting of tonic-clonic activity followed by respiratory arrest and death in all groups. There was a progressive increase in the proportion of rats with seizures and death (Fig. 6). This increase was best fitted by a significant sigmoid dose–response curve for seizures ($R^2 = 0.95$ for cocaine, $R^2 = 0.82$ for cocatropine 40 + 20, and $R^2 = 0.86$ for cocatropine 40 + 60, each at $P < 0.01$) and death ($R^2 = 0.90$ for cocaine, $R^2 = 0.93$ for cocatropine 40 + 20, and $R^2 = 0.90$ for cocatropine 40 + 60, each at $P < 0.01$).

Table 3 shows the calculated number of treatments needed to attain 70% of seizures ($TD_{70}$). For seizures or death, the $TD_{70}$ for cocaine alone was significantly different from cocatropine at both dosages ($P < 0.001$).

**Discussion**

Our findings provide the first detailed evidence of a toxic synergistic effect of the two drugs combined on EEG and behavior in rats. Cocatropine, at both dosages tested, showed a synergistic effect on i) seizures and death after either acute or repeated treatment, ii) EEG mean total spectral power, iii) cardiovascular effects and, to a lesser extent, body temperature. No synergism was seen for spontaneous motor activity. The synergism agrees with the findings Dehkordi et al. (9) who reported that topical treatment with cocaine in anesthetized cats pre-treated with atropine increased the incidence of cocaine-induced respiratory arrest to more than double that when cocaine was given alone.
We established the dosages of cocaine and atropine (40 + 20 and 40 + 60) tested here on the basis of the proportion found in human intoxication, adapted to rats; when the drugs were given singly as one dose, they caused no seizure or death. This is in agreement with other reports where the psychostimulant, given acutely by the same route, produced seizures and death in Sprague-Dawley rats, starting from 100 mg/kg (10, 13, 26, 27). However, in our experiments cocaine increased spontaneous motor activity, body temperature, MAP, and HR, indicating a certain efficacy. Atropine too, at the dose employed, had no toxic effect. However, the same dose counteracted soman-induced intoxication in the same strain (28). The toxic effect of cocatropine on seizures and lethality was confirmed after repeated treatment when the binge paradigm, more representative of the binge pattern of abuse followed by cocaine users (29), was adopted. The two dosages of cocatropine appeared similar in their ability to produce seizures and death, while cocaine alone was less toxic, as shown by

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Seizures (TD70 ± S.E.M.)</th>
<th>Lethality (TD70 ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td>40</td>
<td>5.30 ± 0.58</td>
<td>8.60 ± 0.49</td>
</tr>
<tr>
<td>Cocatropine</td>
<td>40 + 20</td>
<td>2.41 ± 0.14</td>
<td>3.82 ± 0.13</td>
</tr>
<tr>
<td>Cocatropine</td>
<td>40 + 60</td>
<td>3.12 ± 0.42</td>
<td>4.21 ± 0.42</td>
</tr>
</tbody>
</table>

*P<0.001, compared with cocaine alone (Student’s t-test). N = 10 per group.
the larger number of treatments needed to reach 50% of seizures and death.

As regards to the mechanism, it is well known that cocaine produces its toxic effects and a variety of other physiological and behavioral effects through interaction with several distinct CNS binding sites: it inhibits neuronal re-uptake of dopamine, norepinephrine, and serotonin (1) and interacts with both sigma and muscarinic cholinergic receptors (12, 30).

Seizure is a major CNS toxic effect of cocaine and other local anesthetics (31). Blockade of dopamine re-uptake and the resulting elevation of excitatory transmitters have been investigated (32). Studies using pharmacological manipulations of serotonergic neurotransmission systems suggest that 5HT₂ receptors are directly involved in cocaine seizures (12). In addition, the binding affinity of cocaine-like drugs at sigma- or M₁, muscarinic–receptors is inversely correlated with convulsant properties.

Atropine raised the proportion of animals showing seizures and death, after either acute or binge treatment. The anticholinergic syndrome produced by atropine or scopolamine is characterized by symptoms very similar to those during the sympathomimetic syndrome produced by cocaine, as previously reported (8). Agitation, tremors, seizures, tachycardia, hypertension, mydriasis, and altered mental state were the main symptoms. Atropine had no effect on cocaine-induced lethality in mice (10) and was equipotent at M₁- and M₂-muscarinic receptors in the heart and brain (30), suggesting that cocaine may act as an antimuscarinic agent, particularly at high, toxic doses.

The pro-convulsant effect of cocatropine was confirmed by EEG findings where the combination of the two drugs produced a continuous high-amplitude, high-frequency EEG pattern in awake rats. Thus, the mean total spectral power attributable to seizure activity was significantly higher than in cocaine- or atropine-treated groups. Cocaine i.v. typically produced burst epileptiform activity that corresponded to generalized seizure activity in rats (33, 34). Thus, the EEG pattern in cocatropine-treated rats resembles that obtained with toxic doses of cocaine. The finding that cocaine per se induced only a slight, not significant decrease in mean total spectral power is consistent with other reports that a dose unable to produce seizures (30 mg/kg) reduced EEG mean total spectral power (35).

Atropine per se, at the lowest dose, slightly increased mean total spectral power, though not significantly. The anticholinergic drug (1 – 100 mg/kg) did in fact produce synchronization in the neocortical EEG pattern characterized by slow-wave, high-voltage activity (36, 37).

As expected, cocaine and atropine at both dosages increased horizontal motor activity counts. The fact that atropine, in our experiments, was less active seems to agree with the findings of Katz et al. (38) in mice. The combination of the two drugs led to hyperlocomotion similar in intensity to cocaine alone only in non-seizuring rats. This too agrees with Katz et al. (38) who found that pre-treatment with atropine (10 and 30 mg/kg) did not alter the hyperlocomotion induced by cocaine (10 mg/kg). The lack of hyperlocomotion in rats showing seizures was probably due to their general malaise.

As regards to temperature, cocatropine had a significant hyperthermic effect, slightly greater than that in rats given cocaine and atropine (60 mg/kg) alone. The increase of body temperature with cocaine and atropine, given singly, has been reported in rats (39 – 41) and humans (42 – 44). Hyperthermia is a marker of severe toxicity and is associated with a number of complications, including renal failure, disseminated intravascular coagulation, acidosis, hepatic injury, and rhabdomyolysis. However, animals given cocatropine (40 + 20 and 40 + 60) and seizing were less hyperthermic than those not seizing were hyperthermic. During the lethal episode of status epilepticus produced by cocaine, strong, sustained contractions of voluntary muscles, including respiratory movements, hemodynamic dysfunction, and the rapid death of animals have been reported (45). Thus, the cocatropine-induced hypothermia may be part of the terminal event before respiratory arrest and death. The fact that animals treated with cocatropine (40 + 20) were less hyperthermic than those treated with cocatropine (40 + 60) may be due to the greater intensity of seizures observed in the latter group.

The toxicity of cocatropine was confirmed by cardiovascular findings that indicated greater hemodynamic dysfunction than with cocaine alone. Cocaine significantly raised MAP and had a biphasic effect on HR in anesthetized rats, within 30 min. These findings appear to agree with reports with central (11) and peripheral cocaine administration in rats and humans (45, 46). Cocatropine did not change the magnitude of the mean MAP and HR responses to cocaine, but it halved the duration of the pressor response and accentuated the secondary bradycardiac response, suggesting a synergistic effect. A similar effect was reported by Buccafusco et al. (12) who found that pre-treatment with intracisternal atropine accentuated the secondary fall in HR produced by cocaine injected by the same route.

The mechanism of this effect is not clear. Generally, cocaine at stimulating doses increases HR and MAP through a β-adrenergic mechanism, as it can be blocked by propranolol (47), but this effect is counterbalanced by
the homeostatic role of parasympathetic innervation. However, the antimuscarinic action of high doses of cocaine might prevent vagal inhibition of HR and MAP, leaving its sympathomimetic actions unopposed (30). On adding atropine to cocaine, this cardiovascular effect might be enhanced, leading to greater toxicity because atropine, inhibiting the homeostatic role of the parasympathetic innervation of the heart, as previously reported (48), potentiated the antimuscarinic actions of cocaine.

Even if it is unlikely that humans would get access to i.v. diazepam at the time of seizure onset, on the basis of our experiments, it antagonized cocatropine toxicity in terms of seizures, lethality, hemodynamic dysfunction, and temperature changes. Thus we suggest the possibility of treating intoxicated patients with an anxiolytic drug. Diazepam's efficacy was demonstrated after animals had begun to develop cocaine-induced seizures, with no mortality in the diazepam-treated group compared with the 20% in cocatropine (40 + 60) rats treated acutely. Seizures stopped within two minutes after administration of diazepam. These findings agree with those of Guinn et al. (49) who found diazepam i.v. prevented seizures and death in monkeys given cocaine by continuous infusion. Isolated case reports in human beings have also noted the success of diazepam in controlling seizures in cocaine-induced intoxication (50, 51).

The block of hyperlocomotion in cocatropine + diazepam-treated rats may be due to diazepam's basic sedative effect (52) since it did in fact reduce the mean number of horizontal and vertical movement counts. The partial antagonism after diazepam in the mean total spectral power of rats given cocatropine (40 + 60) and seizuring, in comparison with the rats not given diazepam, agrees with Derlet and Albertson (14) who showed that diazepam did not totally suppress central seizure activity but influenced peripheral manifestations of cocaine-induced seizures. Diazepam per se lowered body temperature, though not significantly, as previously reported (53) and slightly reduced cocatropine-induced hypothermia. This latter finding supports the notion that cocaine-induced changes in body temperature are not the primary cause of death, as suggested by Tella et al. (45). Cardiovascular changes induced by cocatropine were partially antagonized by diazepam, which per se did not affect MAP and HR, in accordance with Haskins et al. (54).

The mechanism by which diazepam counteracted cocatropine toxicity might be related to its ability to allosterically enhance the actions of GABA_A at its receptor (55). The major inhibitory neurotransmitter GABA receptor/channel complex is a target for cocaine and other local anesthetics and the suppression of GABA may contribute to cocaine-induced seizures (56, 57). In fact, cocaine has been demonstrated to have a direct reversible inhibitory effect on postsynaptic GABA_A receptors, suggesting that the GABA receptor/channel complex is an additional site for cocaine action. Thus, while the block of the re-uptake mechanisms for dopamine has been regarded as responsible for cocaine's euphorogenic effects (58), cocaine's effect on GABA_A receptor is probably mainly related to its toxic effect.

Other studies, however, have not confirmed that the GABA pathway is involved in cocaine's action on seizure activity (32, 59).

When the diagnosis of anticholinergic poisoning is determined, physostigmine, the reversible inhibitor of acetylcholinesterase, should be considered. However, its use should be avoided due to its adverse effects, including dysrythmias and status epilepticus (60).

In conclusion, even if the mechanism of action must still be elucidated, these findings provide, for the first time, detailed evidence of a synergistic toxic effect of cocatropine when given acutely or repeatedly to rats in proportions similar to those encountered in cases of human intoxication, on quantitative behavioral, EEG, and physiological correlates. Diazepam appears to be an antidote against this toxicity, suggesting a useful potential strategy for dealing with cocatropine-related hospital emergencies.

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