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Antidotal effects of buprenorphine on the behavioral alterations accompanying cocaine and combined cocaine–ethanol toxicity

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Abstract

The present study examined the effects of buprenorphine (BUP), a mixed opioid agonist–antagonist, on the behaviors accompanying cocaine (COCA) and combined cocaine–ethanol (EtOH) toxicity in the surviving mice. Using the activity-counting instrument Supermex, the relationship between the toxic signs and the corresponding behavioral alterations could be assessed. In the COCA-only group, a prolonged increase in the activity counts was caused by a high dose of COCA (75 mg/kg ip). Furthermore, this COCA-induced hyperactivity included ataxic behaviors that were accompanied by visible toxic signs, which were not observed in the mice with no drug treatment. A depressive dose of EtOH (3 g/kg ip) did not significantly modify the mortality rate in the COCA-only group in spite of its anticonvulsant effects. However, the peak activity counts in the survivors were attenuated in the COCA–EtOH group as compared to the COCA-only group. BUP attenuated the mortality rate in both COCA and COCA–EtOH groups, even without any anticonvulsant effects, but the most effective dose differed between the COCA (BUP: 0.25 mg/kg ip) and COCA–EtOH (BUP: 0.5 mg/kg ip) groups. At these BUP doses, the prolonged suppression of the morbid hyperactivity in the COCA–BUP group and the restoration of normal behavior in the COCA–EtOH–BUP group both seemed to be correlated with a good prognosis in the survivors; there was an early recovery from an increased blood pressure (BP), increased heart rate (HR) and decreased respiratory rate (RR) in the COCA–BUP group, and an early recovery from a decreased BP, decreased HR and decreased RR in the COCA–EtOH–BUP group.

Keywords: Cocaine; Ethanol; Buprenorphine; Activity count; Toxicity; Seizure; Physiological function

1. Introduction

In estimating the severity of drug-induced toxicity, the behaviors of intoxicated animals and humans can be used as an important index (Klaassen, 1996). For example, unusual depression usually suggests severe toxicity with a poor prognosis. Increased activity such as violent, panic-like movements accompanied by convulsions also suggests severe and fatal toxicity due to psychostimulants like cocaine (COCA) (Gasior et al., 1999; Klaassen, 1996). COCA is known to cause abnormal behaviors like hyperactivity, even at doses which do not cause fatality, and drugs which can normalize these behavioral alterations have been reported to be useful in the treatment of COCA abuse and toxicity (DiGregorio, 1990; O'Brien, 1996).

The mixed opioid μ agonist–κ antagonist buprenorphine (BUP) is one of these therapeutic drugs which can normalize some of the COCA-induced behavioral alterations (Comer et al., 1993; Mannelli et al., 1993; Winger et al., 1992). A relationship between the effects of COCA and opioid μ and κ receptors has been suggested in previous studies on opioid receptor ligands (Kantak et al., 1999; Suzuki et al., 1992). Furthermore, BUP protected against the fatal toxicities caused by high doses of COCA (Shukla et al., 1991; Witkin et al., 1991). However, the effects of BUP on the toxic effects of high doses of COCA have been evaluated mainly by visible toxic signs (Hayase et al., 1998; Shukla et al., 1991; Witkin et al., 1991) and have not been previously investigated by a quantitative and systematic analysis of both behavioral and physiological alterations, including alterations during the process of recovery.

In the case of toxicity induced by a single dose of COCA, the severity of the COCA toxicity and the effectiveness of BUP can be predicted based on the severity of the abnormally increased activity. However, judging from the toxic effects of

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combining COCA with ethanol (EtOH), a depressant which is frequently coabused with COCA, the suppression of these COCA-enhanced behaviors is not always accompanied by an attenuation of the net toxic effects (Meehan and Schechter, 1995; Randall, 1992). In fact, EtOH has enhanced the lethal effects of COCA in some experiments (Hayase et al., 1996b; Meehan and Schechter, 1995). Interestingly, BUP has been reported to antagonize some of the effects of EtOH (June et al., 1998; Martin et al., 1983), and has also protected against the fatal toxicities caused by the COCA–EtOH combination in our preliminary study (Hayase et al., 1996b). Although the effects of EtOH have been reported to be correlated with opioid μ and κ receptors (Herz, 1997; Matsuzawa et al., 1999), the effects of BUP against combined COCA–EtOH toxicity have not been sufficiently investigated from the viewpoint of behavioral changes.

Therefore, the present study tried to analyze the behavioral changes accompanying the COCA-induced toxic symptoms, and compared the effects of EtOH and/or BUP on these behaviors versus their effects on the toxic physiological symptoms. It may be difficult to examine the behavioral effects of high doses of COCA which can cause fatal toxicities, and the analysis of behaviors becomes impossible for dead animals. However, it is possible that different trends in the behaviors can be observed between the groups of animals with a high mortality rate and the groups of animals with a low mortality rate. Furthermore, it is possible that the behaviors of the survivors during the process of recovery may be modified depending on the increasing severity of the toxic effects. Therefore, the present study examined the tendencies in the survivors' behaviors that seemed to correlate with the prognosis. In order to differentiate these tendencies, an evaluation of the small behavioral changes accompanying the toxic symptoms (e.g., cardiovascular and respiratory symptoms), in addition to gross observable behaviors like ambulation, seems to be useful. This type of investigation has become feasible since the development of the multichannel activity-counting system Supermex (Masuo et al., 1997; Sugiura et al., 1997), which can count even small movements in all three planes of motion using a high sensitivity, multidirectional infrared sensor. By analyzing the data on the net behavioral modifications of the survivors, the present study tried to differentiate the "bad" behavioral patterns in the group with a high mortality rate from the "good" behavioral patterns in the group with an attenuated mortality rate. Furthermore, we discussed valid methods of evaluating treatment efficacy against COCA toxicity based on the data on the BUP treatment.

2. Materials and methods

2.1. Animals and drug treatments

Male ICR mice (60–90 days old) (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) were housed in a forced-air facility maintained at 23°C and 50% relative humidity, with a 12:12 h light/dark cycle (Boyer and Petersen, 1992). The mice were kept individually in single cages (23.5 × 16.5 × 12 cm) with woodchip bedding, and were allowed water and laboratory chow type MF (Oriental Yeast, Tokyo, Japan) ad libitum. The experiments described in this report were conducted in accordance with the "Guidelines for Animal Experiments" of our institution (Committee on Care and Use of Laboratory Animals of Kyoto University, 1988), which are based on the National Institutes of Health Guide for Care and Use of Laboratory Animals. Following these guidelines, if evident and continuous symptoms of pain were caused by high doses of COCA, the experiment was stopped and the mouse was euthanized.

Cocaine hydrochloride (COCA) (Takeda Chemical Industries, Osaka, Japan) was dissolved in saline (Otsuka Pharmaceutical, Tokyo, Japan), and was administered by an intraperitoneal (ip) injection at 75 mg/kg body weight in a volume of 5 ml/kg. This dose of COCA was selected from the doses at which severe toxic signs were observed (Hayase et al., 1996b). However, high doses of COCA without any interaction with BUP in preliminary administration studies were avoided. Interactions between even low doses of COCA and BUP have been previously reported (Comer et al., 1993; Mannelli et al., 1993; Winger et al., 1992). However, the present study focused on the toxic signs, and from this viewpoint the dose that provided the most notable interaction with BUP in preliminary trials (75 mg/kg) was selected. The COCA was injected 15 min after an intraperitoneal injection of saline (COCA-only group) or 3 g/kg ethanol (EtOH) (Nacalai Tesque, Kyoto, Japan) (COCA–EtOH-only group), in a total volume of 10 ml/kg (diluted with saline for the EtOH solution). This dose of EtOH was selected from the depressive but nonfatal doses which had displayed some interactions with COCA, such as enhancing the COCA-induced physiological effects and suppressing the COCA-stimulated locomotor activity (Boyer and Petersen, 1992; Meehan and Schechter, 1995). In the BUP-cotreated groups, buprenorphine hydrochloride (Otsuka Pharmaceutical), which was diluted with saline to the most effective dose, was given 15 min before the above saline vehicle (COCA–BUP group) or EtOH (COCA–EtOH–BUP group) injection. This dose of BUP was selected from nontoxic doses based on previous studies (Hayase et al., 1996b; Shukla et al., 1991; Witkin et al., 1991) and some preliminary experiments. The most effective dose of BUP for attenuating the mortality rate was then chosen, even though that dose was different between the COCA–BUP and COCA–EtOH–BUP groups. The intraperitoneal dose of BUP used was 0.25 mg/kg in the COCA–BUP group, and 0.5 mg/kg in the COCA–EtOH–BUP group, in a volume of 5 ml/kg. In the COCA-only and COCA–EtOH-only groups, 5 ml/kg of saline vehicle was injected instead of BUP.

2.2. Scoring of the toxic signs

In all administration groups, the severity of the convulsive seizures and the visible toxic signs (respiratory distress and locomotive disturbance) were monitored and scored for the surviving mice. The total number of mice examined was 15 in the COCA–EtOH-only group and 10 in the other groups. The data on the severity of the convulsive seizures and the visible toxic signs were collected only from the surviving mice (Tables 1 and 2). For the mortality rate, the percentage of dead mice at 24 h after the drug administration was recorded; all of the deaths had occurred by this time point in our previous studies (Hayase et al., 1996a,b). In the present study, all of the deaths had occurred by the 5-h time point after the drug treatment.

The severity of the convulsive seizures was scored based on the methods described in a previous study (Przewlocka et al., 1994). The observation period for the seizures was 24 h, and the most severe episode for each mouse was evaluated. A score of 0 (absence of convulsions), a score of 1 (short-lasting episodes [< 1 min] of clonic convulsions), a score of 2 (clonic convulsions with a loss of the righting reflex, but less continuous as compared to the score 3 convulsions), a score of 3 (episodic convulsions including serial convulsions lasting longer than 5 min), and a score of 4 (violent convulsions similar to those observed in the dead mice) could be differentiated.

The severity of the visible respiratory distress and locomotive disturbances was scored as 0 (none), 1 (moderate) or 2 (severe and similar to the disorders observed in the dead mice) for the same surviving mice at 1 and 5 h after the drug treatment. These visible signs were based on the severity of the visible disorders (irregularity in the breathing movements, unsteady gait, etc.), regardless of any quantitative alterations (activity counts, etc.).

In order to avoid subjective errors, the behavioral alterations were evaluated independently by three observers. All observers had previously evaluated the behaviors of both normal (no drug treatment) and severely intoxicated mice, and a compromise score was decided after a discussion on the behavioral score for each mouse.

Table 1
Mortality rate and seizure score

	Mortality rate (%)	Seizure score
COCA-only group ($n=10$)	60	3.3 ± 0.4 ($n=4$)
COCA–BUP group ($n=10$)	20[a]	3.3 ± 0.7 ($n=8$)
COCA–EtOH-only group ($n=15$)	73	0.8 ± 0.4[a] ($n=4$)
COCA–EtOH–BUP group ($n=10$)	40[b]	0.7 ± 0.5[a] ($n=6$)

Mortality rate (%) and seizure score (0–4) in the surviving mice from the COCA (75 mg/kg ip)-only (total: $n=10$, survivor: $n=4$), COCA–BUP (0.25 mg/kg ip) (total: $n=10$, survivor: $n=8$), COCA–EtOH (3 g/kg ip)-only (total: $n=15$, survivor: $n=4$) and COCA–EtOH–BUP (0.5 mg/kg ip) (total: $n=10$, survivor: $n=6$) groups. The data on the seizure scores represent means ± S.D.

[a] Significant ($P<.05$) attenuation as compared to the COCA-only group.

[b] Significant ($P<.05$) attenuation as compared to the COCA–EtOH-only group.

Table 2
Scores for the visible toxic symptoms

	Respiratory distress		Locomotive disturbance	
	1-h point	5-h point	1-h point	5-h point
COCA-only group ($n=4$)	1.3 ± 0.4	0.5 ± 0.5	1.5 ± 0.5	0.8 ± 0.4
COCA–BUP group ($n=8$)	0.6 ± 0.5[a]	0.0 ± 0.0	1.0 ± 0.7	0.3 ± 0.4[a]
COCA–EtOH-only group ($n=4$)	1.3 ± 0.4	0.5 ± 0.5	2.0 ± 0.0	1.0 ± 0.0
COCA–EtOH–BUP group ($n=6$)	0.8 ± 0.4[a,b]	0.2 ± 0.4	1.3 ± 0.5[b]	0.5 ± 0.5[b]

Scores (0–2) for the visible toxic signs (respiratory distress and locomotive disturbances) at 1 and 5 h after the drug treatment for the same surviving mice shown in Fig. 1. In order to avoid subjective errors, the behavioral alterations were independently evaluated by three persons as described in the text, and compromise scores are shown. The data represent means ± S.D. In all groups, the score at the 5-h time point was significantly ($P<.05$) lower than at the 1-h point (not shown in this table).

[a] Significant ($P<.05$) attenuation as compared to the COCA-only group.

[b] Significant ($P<.05$) attenuation as compared to the COCA–EtOH-only group.

2.3. Evaluation of the locomotor activity alterations

At the same time as the observation of the toxic signs, the locomotor activity was evaluated by the multichannel activity-counting system Supermex (Muromachi Kikai, Tokyo, Japan) (Masuo et al., 1997; Sugiura et al., 1997). This instrument can monitor even minute movements in all three planes of motion (sagittal, coronal and horizontal) as one movement, since its infrared sensor with multiple Fresnel lenses that can be moved close enough to the cage can capture multidirectional locomotor alterations in a single mouse. The Supermex instrument was connected to a behavioral analyzing system (CompACT AMS) (Muromachi Kikai), which can interpret each movement as one count. Therefore, vertical movements such as jumping, as well as horizontal movements such as walking and running, could be counted. Furthermore, small movements of the limbs, head and tail accompanying the shaking, spasms and twitching, which become frequent due to the COCA-induced seizures, could also be recorded as activity counts within the discrimination capacity of the sensor. The number of activity counts was noted whenever each movement, as an infrared-ray signal, crossed the multidirectional lens-equipped antennae of the sensor, which covered all directions in the cage space. Therefore, the number of activity counts increased proportionally corresponding to the increased temporal and spatial activity of each mouse. The counting was performed continuously, and the data were recorded every 20 min. The locomotor activity was monitored for all mice, but the data were analyzed only for the surviving mice. The time course

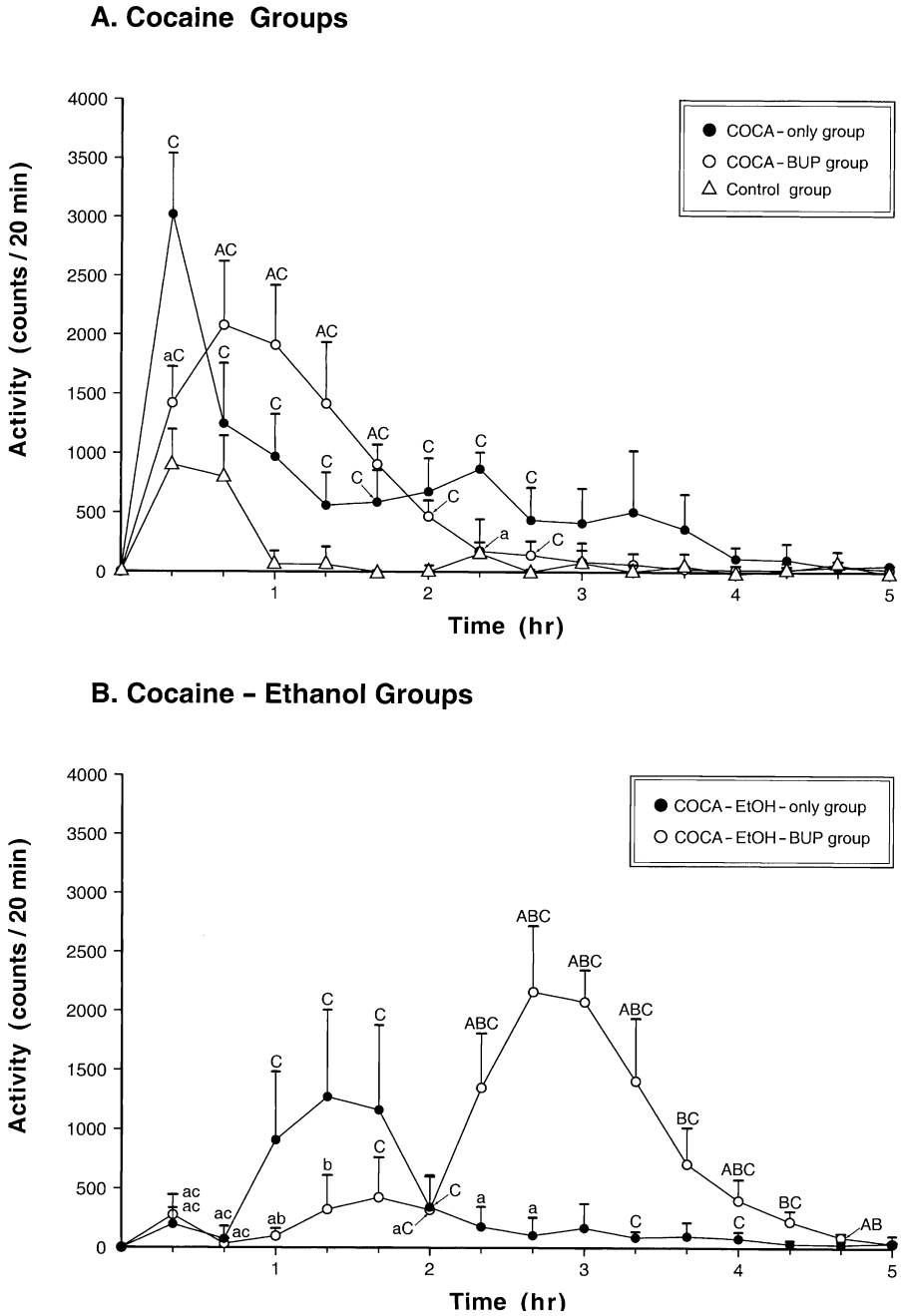

Fig. 1. Time course of the motor activity counts for the surviving mice in the COCA-only ($n=4$) (A), COCA–BUP ($n=8$) (A), COCA–EtOH-only ($n=4$) (B) and COCA–EtOH–BUP ($n=6$) (B) groups. The data from a nontreatment control group of mice ($n=5$) are also shown (A). All of the abbreviations and the drug doses used are explained in Table 1. For each time point, the differences were noted in the same manner as per the tables.

for the surviving mice is shown in Fig. 1. The time course data for a nontreatment control group of mice ($n=5$) (Fig. 1A) were also obtained by the Supermex instrument under the same conditions (e.g., experiment time etc.), and the effects of factors other than the drugs (e.g., Supermex environment) were also considered. Each 20-min activity count value at each time point was compared between the groups. Furthermore, in order to evaluate and compare the more continuous stimulatory or depressive effects of each drug, the mean value of each 1-h serial count was calculated based on the above time course data (Fig. 1), and then the largest and the smallest 1-h count values were obtained and compared between the groups.

2.4. Evaluation of physiological function

As physiological parameters, the blood pressure (BP), heart rate (HR) and respiratory rate (RR) at the 1- and 5-h

time points were also recorded. The evaluation of these parameters could not be performed simultaneously with the activity recording because it disrupted the activity counting system. Therefore, the examination was performed for the other groups of surviving mice undergoing the same drug treatment. The data from 4 surviving mice in each group were collected, but the total number of mice examined for obtaining the 4 survivors was 10 in the COCA-only group, 5 in the COCA–BUP group, 15 in the COCA–EtOH-only group, and 7 in the COCA–EtOH–BUP group (these total numbers are not shown in Table 4). The BP of the celiac artery was monitored by an automatic monitor (type EW280) (Matsushita Electronics, Hikone, Japan). Both the systolic and diastolic blood pressures (SBP and DBP) could be monitored with this instrument. The HR was derived from the pulsatile pressure recorded as beats per minute (Pitts et al., 1987). The RR (breaths per minute) was monitored by visual observation of the diaphragm movement (Pitts et al., 1987). For all of these parameters, data from nontreatment control mice ($n=5$) were also obtained.

2.5. Statistical analysis

The chi-square test was used to compare the mortality rate (%) (Shukla et al., 1991). For other values, the data in all groups were subjected to a one-way analysis of variance (ANOVA), and then two sample t tests with Welch's correction were performed for all necessary combinations of the two groups in order to evaluate the effects of each drug (Shukla et al., 1991). All of the comparisons were performed using a statistical software package and its manual (Shakai Johou Service, 1993). Unless otherwise noted, P values less than .05 were considered to be statistically significant.

3. Results

3.1. Mortality rate and toxic signs

The mortality rate (Table 1) exceeded 50% in both the COCA-only (60%) and the COCA–EtOH-only (73%) groups, and was slightly increased in the latter group. BUP attenuated the mortality rate in both the COCA group (60 → 20%) and the COCA–EtOH group (73 → 40%). All of the deaths had occurred by 5 h after the drug administration, but most of the deaths (10 out of 11 deaths) in the COCA–EtOH-only group occurred after the time point of the peak activity counts.

The seizure scores in the survivors were significantly attenuated by EtOH, but were not significantly altered by BUP (Table 1).

With respect to the visible respiratory distress in the surviving mice (Table 2), the scores at the 1-h time point were significantly higher in the non-BUP group than in the BUP-treated groups. At the 5-h time point, a significant recovery of these scores was observed in all groups, but the scores were still slightly higher in the non-BUP groups as compared to the BUP-treated groups. For the visible locomotive disturbances (Table 2), the score at the 1-h time point was not significantly different between the COCA-only and the COCA–BUP groups. However, the score in the COCA–EtOH group was significantly higher than in the COCA–EtOH–BUP group. At the 5-h time point, despite the recovery of the scores in all groups as compared to the 1-h time point, a slight but significantly more severe locomotive disturbance could still be observed in the non-BUP groups as compared to the BUP-treated groups.

3.2. Locomotor activity alterations

The time courses of the motor activity counts for the surviving mice and the control mice are shown in Fig. 1. The counts for the first 5 h are shown, because no mice died after that time point in the present experiment. In all groups, the data for the largest and the smallest 1-h counts are shown in Table 3. Slightly increased activity was observed in the control group immediately after they entered the Supermex instrument. However, even in the EtOH-cotreated group, the largest 1-h counts were significantly increased as compared to the control group (Table 3). In contrast, the smallest 1-h counts were significantly increased in the COCA-only and the COCA–EtOH–BUP groups as compared to the control group. In the COCA–BUP group ($n=8$), although some heterogeneity in the values was observed (Fig. 1A), the largest 1-h counts in the survivors were not significantly altered by the single BUP cotreatment, even though the mortality rate was attenuated as compared to the COCA-only group ($n=4$) (Tables 1 and 3). However, BUP did significantly attenuate the smallest 1-h counts in the COCA–BUP group as compared to the COCA-only group (Table 3). In the COCA–EtOH-only group ($n=4$), EtOH significantly attenuated both the largest

Table 3
Largest and smallest 1-h activity counts

	Largest 1-h counts	Smallest 1-h counts
Control group ($n=5$)	1821 ± 552	21 ± 18
COCA-only group ($n=4$)	5240 ± 706^{a}	107 ± 30^{a}
COCA–BUP group ($n=8$)	5634 ± 1102^{a}	39 ± 37^{b}
COCA–EtOH-only group ($n=4$)	$3531 \pm 924^{a,b}$	43 ± 21^{b}
COCA–EtOH–BUP group ($n=6$)	$5974 \pm 704^{a,c}$	$356 \pm 125^{a,c,d}$

Largest and smallest 1-h counts for the same mice from Fig. 1. The data were obtained from 1-h serial values in Fig. 1, and represent means ± S.D.

[a] Significant ($P<.05$) increase as compared to the control group.

[b] Significant ($P<.05$) attenuation as compared to the COCA-only group.

[c] Significant ($P<.05$) increase as compared to the COCA–EtOH-only group.

[d] Significant ($P<.05$) increase as compared to the COCA-only group.

and the smallest 1-h counts as compared to the COCA-only group (Table 3). In the COCA–EtOH–BUP group ($n=6$), apart from the significant alterations due to the shift in the time course curve (Fig. 1B), BUP significantly increased both the largest and the smallest 1-h counts in the survivors as compared to the COCA–EtOH-only group, corresponding to the attenuation in the mortality rate (Tables 1 and 3). Therefore, a delayed and prolonged increase in the activity counts was observed in the COCA–EtOH–BUP group during the process of recovery.

3.3. Physiological function

The BP, HR and RR data for the four surviving mice that were obtained by the above process are shown in Table 4. At the 1-h time point (Table 4A), the SBP was significantly increased in the COCA-only group and was significantly decreased in the COCA–EtOH-only group, as compared to the control group. The HR increase in the COCA-only group, the HR attenuation in the COCA–EtOH-only group and the RR attenuation in both groups were also significantly different as compared to the control group. In the BUP-treated groups, a BUP-induced amelioration of each parameter was observed, and the values were not significantly different from those in the control group, except for an attenuated RR value in the COCA–EtOH–BUP group. At the 5-h time point (Table 4B) in all administration groups, both the SBP and DBP had recovered to values which were not significantly different from the control. However, an attenuation of the HR (COCA–EtOH-only group) and RR (COCA-only and COCA–EtOH-only groups) could still be observed in the non-BUP groups, even though they were not statistically different from the control group. A single dose of EtOH (3 g/kg) tended to attenuate the BP, HR and RR in a preliminary experiment, but no significant alterations were observed (data not shown).

Table 4
Cardiovascular and respiratory parameters

	BP			
	SBP	DBP	HR	RR
(A) 1-h time point				
Control group	109.7 ± 10.1	76.2 ± 7.7	533 ± 32	125 ± 15
COCA-only group	121.5 ± 7.7[a]	79.6 ± 6.3	583 ± 38[a]	103 ± 10[b]
COCA–BUP group	114.5 ± 7.6	76.1 ± 6.8	550 ± 42	116 ± 17
COCA–EtOH-only group	95.2 ± 10.0[b,c]	68.2 ± 5.8[c]	467 ± 42[b,c]	100 ± 11[b]
COCA–EtOH–BUP group	104.8 ± 9.4[c]	69.8 ± 7.6[c]	499 ± 47[c]	109 ± 12[b]
(B) 5-h time point				
Control group	112.8 ± 10.2	77.2 ± 8.0	523 ± 28	120 ± 18
COCA-only group	111.9 ± 8.8	75.3 ± 6.2	533 ± 38	108 ± 12
COCA–BUP group	118.2 ± 9.5	76.5 ± 7.4	522 ± 35	125 ± 13[d]
COCA–EtOH-only group	105.7 ± 10.1	73.2 ± 6.1	485 ± 31[c]	105 ± 13
COCA–EtOH–BUP group	108.4 ± 9.8	73.9 ± 6.7	521 ± 36	128 ± 15[d,e]

Alterations in the BP (mm Hg), HR (beats/min) and RR (breaths/min) at the 1- (A) and 5-h (B) time points after drug treatment for the groups of four surviving mice described in the text. The abbreviations are the same as in the text. The data from a nontreatment control group of mice ($n=5$) are also shown. The data represent means ± S.D.

[a] Significant ($P<.05$) increase as compared to the saline control group.

[b] Significant ($P<.05$) attenuation as compared to the saline control group.

[c] Significant ($P<.05$) attenuation as compared to the COCA-only group.

[d] Significant ($P<.05$) increase as compared to the COCA-only group.

[e] Significant ($P<.05$) increase as compared to the COCA–EtOH-only group.

4. Discussion

The present study elucidated some of the characteristic behavioral alterations caused by a toxic COCA dose, with or without BUP and/or EtOH. Since BUP did not antagonize the severe convulsive seizures observed mainly during the early period after drug treatment, neither the early largest 1 h counts nor the visible locomotive disturbances, including the convulsive seizures, were significantly different between the COCA-only and the COCA–BUP groups (Tables 1–3). However, in the surviving mice, the severity of the COCA-induced toxic effects and the BUP-induced antidotal effects could be estimated by observing the process of recovery in the activity counts; this could be accomplished by monitoring all of the locomotor activity, including the small movements of all parts of the body, with the Supermex system. Even in the surviving mice from the COCA-only group, small morbid behaviors which seemed to be correlated with respiratory and/or cardiovascular dysfunction (Table 4) could be continuously monitored throughout the 5-h period (Fig. 1A and Table 3). BUP suppressed these small behaviors, which corresponded to the attenuation in the mortality rate (Table 1) and the improvement in the physiological data (Table 4) in the COCA–BUP group as compared to the COCA-only group.

On the other hand, in the COCA–EtOH-only group, the early activity counts including the peak counts (Fig. 1B and Table 3) were still increased as compared to the control group, but were attenuated as compared to the COCA-only group. This behavioral suppression included an attenuation of the normal behaviors that seemed to be correlated with respiratory and cardiovascular dysfunction (Table 4). In the COCA–EtOH–BUP group, unlike the COCA–BUP group, the activity counts were increased (Table 3). These different effects might be due to the different doses of BUP (0.5 vs. 0.25 mg/kg) used. However, the attenuation of the mortality rate (Table 1), the amelioration of the visible locomotive disturbances (Table 2) and the improvement in the physiological data (Table 4) were present in both the COCA–BUP and the COCA–EtOH–BUP groups. At doses of 0.25 and 0.5 mg/kg, the

increase in the blood COCA concentration due to BUP and the related enhancement of the toxic effects have not been reported to be significant, with or without the EtOH cotreatment (Hayase et al., 1996b). However, the slightly prolonged increase in the behavioral counts, which was observed in the COCA–BUP group (Fig. 1A), might be due to the BUP-induced small kinetic alterations. In the COCA–EtOH–BUP group, the effects of BUP against COCA toxicity seemed to be attenuated due to the BUP–EtOH interaction, as was suggested in some reports (June et al., 1998; Martin et al., 1983), and higher doses of BUP (0.5 mg/kg) seemed to be more effective due to this attenuated influence of BUP on the COCA effects. Although the blood COCA concentration had not been reported to be altered significantly by 0.5 mg/kg BUP in the COCA–EtOH group (Hayase et al., 1996b), the shift in the time course curve of the activity counts was observed in the COCA–EtOH–BUP group (Fig. 1B). This phenomenon seemed to be correlated with the prolonged but weakened influence of EtOH due to BUP that was not correlated with the blood COCA levels (Hayase et al., 1996a, 1998), and the attenuation in the level of the toxic COCA–EtOH metabolite (Hayase et al., 1996b; Hearn et al., 1991a,b; Randall, 1992).

Judging from the prolonged increase in the activity counts (Fig. 1B and Table 3) and its previously reported interaction with EtOH (Hayase et al., 1998; June et al., 1998; Martin et al., 1983), BUP might have antagonized the delayed toxic depressive effects of EtOH. Due to this prolonged increase, the attenuation of the behavioral counts due to sleep (e.g., the smallest 1-h counts), which was observed in the control group, was not observed in the survivors in the COCA–EtOH–BUP group during the 5-h period. This phenomenon might exert a deleterious effect in the long term, even though the morbid behaviors were not increased and the prognosis itself was ameliorated by BUP. However, from our observation, it can be concluded that BUP, by causing both stimulatory and inhibitory effects on the behavioral counts depending on its dosage, antagonized the toxic behavioral effects in both COCA and combined COCA–EtOH groups.

The toxic respiratory and cardiovascular effects of high dose COCA include an attenuated RR, increased BP and an increased HR (O'Brien, 1996; Tseng et al., 1991). EtOH has been reported to enhance these COCA-induced effects (Henning et al., 1994; Uszenski et al., 1992), and the fatal toxic symptoms have also been suggested to be exacerbated by EtOH due to the synthesis of toxic metabolites (Hearn et al., 1991a,b; Randall, 1992) and/or a prolongation of the half-life of COCA (Hedaya and Pan, 1996, 1997). In the present experiment, no significant increase in the mortality rate was observed in the COCA–EtOH group as compared to the COCA-only group (Table 1). However, the toxic cardiovascular depression due to the combined high-dose EtOH (3 g/kg) seemed to cause symptoms that were different from those caused by the COCA-only treatment. Corresponding to the attenuated behavioral counts (Fig. 1B and Table 3) and increased locomotive disturbances (Table 2), an attenuation of the BP, HR and RR could be observed in the EtOH groups as compared to the control group (Table 4). The effects of BUP in the COCA–EtOH–BUP group were not limited to a simple delay of the process of COCA-induced stimulation and EtOH-induced depression, as might be predicted from the graph in Fig. 1. Corresponding to the delayed increase in the activity counts (Fig. 1B), a recovery from the locomotive disturbances (Table 2) could be observed, and the physiological parameters had returned to normal earlier than in the non-BUP group (Table 4). Therefore, the recovery from the toxic symptoms seemed to coincide with the behavioral effects (an increase in normal behavior, etc.) in the EtOH groups, as well as in the non-EtOH groups.

The problem with BUP as an antidote against COCA toxicity is the absence of any anticonvulsant activity. Severe, convulsive seizures accompanied the COCA-induced toxicity regardless of BUP cotreatment. However, the behavioral analysis performed at the present doses could distinguish one advantage for BUP that may compensate for its lack of anticonvulsant activity: there were no depressive side effects on the respiratory and cardiovascular systems caused by BUP that were observed in the COCA–EtOH group. Furthermore, any unfavorable side effects accompanying the COCA-induced seizures were not observed in the respiratory and cardiovascular systems in the COCA–BUP group (Table 4), despite the presence of severe convulsive seizures (Table 1). Many anticonvulsants, including EtOH, have strong depressive effects on the respiratory and cardiovascular systems (Hobbs et al., 1996; McNamara, 1996). Even far less toxic anticonvulsant drugs with EtOH-like GABA receptor-related effects have been reported to cause such toxic effects (Hobbs et al., 1996; McNamara, 1996). Since BUP is known to function as a mixed μ agonist–κ antagonist, its protective effects may be correlated with opioid receptor-mediated neuroprotective effects, which could influence the respiratory and cardiovascular systems (Faden, 1996; Jones and Ross, 1995). Therefore, this drug could provide useful antidotal effects in a clinical setting (Mello et al., 1993; Rothman et al., 1995). BUP has been reported to either attenuate (Comer et al., 1993; Mannelli et al.,1993; Winger et al., 1992) or increase (Brown et al., 1991; Kuribara and Tadokoro, 1991) the behavioral effects of COCA. Similar dual interactions have been observed for the BUP–EtOH combination (June et al., 1998; Kuribara et al., 1991; Martin et al., 1983). These dual effects may be caused due to the mixed functions of BUP and may be closely correlated with the neuroprotective effects, but the precise roles of μ and κ actions have not been elucidated. Nevertheless, judging from the present results, it can be concluded that the mixed opioid drugs like BUP, with their balanced activity, consistently increased the normal behaviors and suppressed the morbid behaviors, and could also

normalize the respiratory and cardiovascular systems, even in the combined COCA–EtOH group with complicated mechanisms underlying its toxic effects. This supports the usefulness of these drugs in the treatment of both COCA and combined COCA–EtOH toxicity.

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References

Boyer CS, Petersen DR. Enzymatic basis for the transesterification of cocaine in the presence of ethanol: evidence for the participation of microsomal carboxylesterases. J Pharmacol Exp Ther 1992;260:939–46.

Brown EE, Finlay JM, Wong JT, Damsma G, Fibiger HC. Behavioral and neurochemical interactions between cocaine and buprenorphine: implications for the pharmacotherapy of cocaine abuse. J Pharmacol Exp Ther 1991;256:119–26.

Comer SD, Lac ST, Curtis LK, Carroll ME. Effects of buprenorphine and naltrexone on reinstatement of cocaine-reinforced responding in rats. J Pharmacol Exp Ther 1993;267:1470–7.

Committee on Care and Use of Laboratory Animals of Kyoto University. Guidelines for animal experiments Kyoto: Kyoto University Council, 1988 (in Japanese).

DiGregorio GJ. Cocaine update: abuse and therapy. Am Fam Physician 1990;41:247–50.

Faden AI. Neurotoxic versus neuroprotective actions of endogenous opioid peptides: implications for treatment of CNS injury. NIDA Res Monogr 1996;163:318–30.

Gasior M, Ungard JT, Witkin JM. Preclinical evaluation of newly approved and potential antiepileptic drugs against cocaine-induced seizures. J Pharmacol Exp Ther 1999;290:1148–56.

Hayase T, Yamamoto Y, Yamamoto K, Fukui Y. Role of brain cocaethylene levels in combined cocaine–ethanol lethality in mice. Jpn J Alcohol Drug Depend 1996a;31:95–109.

Hayase T, Yamamoto Y, Yamamoto K. Protective effects of buprenorphine against amplified cocaine and ethanol lethality in mice: role of cocaethylene. J Toxicol Sci 1996b;21:143–56.

Hayase T, Yamamoto Y, Yamamoto K, Abiru H, Fukui Y. Effects of buprenorphine and Ro 15-4513 on delayed death and brain beta-endorphin levels in rats treated with cocaine or cocaine–ethanol. Jpn J Alcohol Drug Depend 1998;33:112–34.

Hearn WL, Rose S, Wagner J, Ciarleglio A, Mash DC. Cocaethylene is more potent than cocaine in mediating lethality. Pharmacol Biochem Behav 1991a;39:531–3.

Hearn WL, Flynn DD, Hime GW, Rose S, Cofino JC, Mantero-Atienza E, Wetli CV, Mash DC. Cocaethylene: a unique cocaine metabolite displays high affinity for the dopamine transporter. J Neurochem 1991b;56:698–701.

Hedaya MA, Pan WJ. Cocaine and alcohol interactions in naïve and alcohol-pretreated rats. Drug Metab Dispos 1996;22:807–12.

Hedaya MA, Pan WJ. Effect of alcohol coadministration on the plasma and brain concentrations of cocaine in rats. Drug Metab Dispos 1997;25:647–50.

Henning RJ, Wilson LD, Glauser JM. Cocaine plus ethanol is more cardiotoxic than cocaine or ethanol alone. Crit Care Med 1994;22:1896–906.

Herz A. Endogenous opioid systems and alcohol addiction. Psychopharmacology 1997;129:99–111.

Hobbs WR, Rall TW, Verdoorn TA. Hypnotics and sedatives; ethanol. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman A, editors. Goodman & Gilman's the pharmacological basis of therapeutics. New York: McGraw-Hill, 1996. pp. 361–98.

Jones MK, Ross DT. The partial mu opiate agonist buprenorphine protects a sub-population of thalamic reticular neurons following cardiac arrest in rats. Neurosci Lett 1995;185:91–4.

June HL, Cason CR, Chen SH, Lewis MJ. Buprenorphine alters ethanol self-administration in rats: dose–response and time-dependent effects. Psychopharmacology 1998;140:29–37.

Kantak KM, Riberdy A, Spealman RD. Cocaine–opioid interactions in groups of rats trained to discriminate different doses of cocaine. Psychopharmacology 1999;147:257–65.

Klaassen CD. Principles of toxicology and treatment of poisoning. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman A, editors. Goodman & Gilman's the pharmacological basis of therapeutics. New York: McGraw-Hill, 1996. pp. 63–76.

Kuribara H, Tadokoro S. The ambulation-increasing effect of buprenorphine in mice: comparison with the effect of morphine. Jpn J Alcohol Drug Depend 1991;26:37–48.

Kuribara H, Asahi T, Tadokoro S. Enhancement of the ambulation-increasing effect of opioid analgesics by ethanol in mice. Jpn J Pharmacol 1991;56:457–63.

Mannelli P, Janiri L, Tempesta E, Jones RT. Prediction in drug abuse: cocaine interactions with alcohol and buprenorphine. Br J Psychiatry 1993;163:39–45.

Martin A, Pilotto R, Singer G, Oei TP. The suppression of ethanol self injection by buprenorphine. Pharmacol Biochem Behav 1983;19:985–6.

Masuo Y, Matsumoto Y, Morita S, Noguchi J. A novel method for counting spontaneous motor activity in the rat. Brain Res Brain Res Protoc 1997;1:321–6.

Matsuzawa S, Suzuki T, Misawa M, Nagase H. Different roles of mu-, delta- and kappa-opioid receptors in ethanol-associated place preference in rats exposed to conditioned fear stress. Eur J Pharmacol 1999;368:9–16.

McNamara JO. Drugs effective in the therapy of the epilepsies. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilaman A, editors. Goodman & Gilman's the pharmacological basis of therapeutics. New York: McGraw-Hill, 1996. pp. 461–86.

Meehan SM, Schechter MD. Premorbid behaviors produced by cocaine, ethanol and cocaethylene in the mouse. Gen Pharmacol 1995;26:99–106.

Mello NK, Mendelson JH, Lukas SE, Gastfriend DR, Teoh SK, Holman BL. Buprenorphine treatment of opiate and cocaine abuse: clinical and preclinical studies. Harv Rev Psychiatry 1993;1:168–83.

O'Brien CP. Drug addiction and drug abuse. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman A, editors. Goodman & Gilman's the pharmacological basis of therapeutics. New York: McGraw-Hill, 1996. pp. 557–80.

Pitts DK, Udom CE, Marwah J. Cardiovascular effects of cocaine in anesthetized and conscious rats. Life Sci 1987;40:1099–111.

Przewlocka B, Lason W, Machelska H, Przewlocka R. The effects of cocaine-induced seizures on the proenkephalin mRNA level in the mouse hippocampus; a possible involvement of the nitric oxide pathway. Neurosci Lett 1994;168:81–4.

Randall T. Cocaine, alcohol mix in body to form even longer lasting, more lethal drug. JAMA, J Am Med Assoc 1992;267:1043–4.

Rothman RB, Ni Q, Xu H. Buprenorphine: a review of the binding literature. In: Cowman A, Lewis JW, editors. Buprenorphine: combatting drug abuse with a unique opioid. New York: Wiley-Liss, 1995. pp. 19–29.

Shakai Johou Service. Manual of medical statistics. Tokyo: Shakai Johou Service, 1993 (in Japanese).

Shukla VK, Goldfrank LR, Turndorf H, Bansinath M. Antagonism of acute cocaine toxicity by buprenorphine. Life Sci 1991;49:1887–93.

Sugiura M, Muraoka S, Yoshizawa T, Watabe K, Murakami O, Yamaguchi F. The application of the measuring apparatus of locomotor activity by

infrared sensor system using multi-Fresnel lenses to forced swimming test. Jpn J Neuropsychopharmacol 1997;19:287–91 (in Japanese with an English abstract).

Suzuki T, Shiozaki Y, Masukawa Y, Misawa M, Nagase H. The role of mu- and kappa-opioid receptors in cocaine-induced conditioned place preference. Jpn J Pharmacol 1992;58:435–42.

Tseng CC, Derlet RW, Stark LG, Albertson TE. Cocaine-induced respiratory depression in urethane-anesthetized rats: a possible mechanism of cocaine-induced death. Pharmacol Biochem Behav 1991; 39:625–33.

Uszenski RT, Gillis RA, Schaer GL, Analouei AR, Kuhn FE. Addictive myocardial depressant effects of cocaine and ethanol. Am Heart J 1992;124:1276–83.

Winger G, Skjoldager P, Woods JH. Effects of buprenorphine and other opioid agonists and antagonists on alfentanil- and cocaine-reinforced responding in rhesus monkeys. J Pharmacol Exp Ther 1992;261: 311–7.

Witkin JM, Johnson RE, Jaffe JH, Grayson NA, Rice KC, Katz JL. The partial opioid agonist, buprenorphine, protects against lethal effects of cocaine. Drug Alcohol Depend 1991;27:177–84.