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Comparison of effect of ethanol and anticonvulsants on cardiovascular drug-modified cocaine toxicity

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Abstract

The anticonvulsant (AC drug)- or ethanol (EtOH)-modified effects of cardiovascular (CV) drugs against cocaine (COCA)-induced toxicity were examined in male ICR mice. Nontoxic doses of the CV drugs nimodipine (NIMO), prazosin (PRA), phentolamine (PHEN), propranolol (PRO), and enalapril (ENA) were used with or without the AC drugs diazepam (DZP), phenobarbital (PHB), phenytoin (PHY), and EtOH. Each CV drug combined with or without each AC drug was administered intraperitoneally (IP) 5 min before an IP injection of COCA 75 mg/kg. Of the CV drugs examined, PRA 5 mg/kg and PHEN 5 mg/kg protected against COCA-induced seizures, but only the α1-adrenergic blocking agent PRA protected against COCA-induced deaths. Of the AC drugs examined, DZP 5 mg/kg and PHB 50 mg/kg, as well as EtOH 3 g/kg, attenuated the severity of the COCA-induced seizures, but only PHB protected against COCA-induced deaths. The total mortality rate was significantly, often synergistically, decreased compared to the COCA-only group when the appropriate CV drugs were combined with the AC drugs: PRA 5 mg/kg in the EtOH-cotreated groups, PRA 5 mg/kg, PHEN 5 mg/kg or ENA 10 mg/kg in the DZP-cotreated groups, and NIMO 5 mg/kg, PRA 5 mg/kg, PHEN 5 mg/kg or PRO 10 mg/kg in the PHB-cotreated groups. The decrease in the COCA concentration in the blood and/or brain was not always accompanied by an attenuation of the mortality rate. However, the attenuation of severe seizures by a single PRA, PHEN, DZP, or PHB cotreatment was accompanied by a decrease in the brain COCA concentration.

Keywords: Cocaine; Seizures; Deaths; Ethanol; Diazepam; Phenobarbital; Phenytoin; Nimodipine; Prazosin; Phentolamine; Propranolol; Enalapril

1. Introduction

Fatal toxicity due to cocaine (COCA) is closely related to its effects as a reuptake inhibitor of catecholamines, including dopamine, with central nervous system (CNS)-stimulating effects [35,38]. Previous studies have suggested that the cardiovascular (CV) effects of COCA, which contribute greatly to fatal COCA toxicity [20], were also closely correlated with CNS-related mechanisms [35,38]. However, some of the CV effects of COCA, such as myocardial ischemia and arrhythmia, seem to be caused by the direct, peripheral sympathomimetic, sodium channel-blocking and/or calcium channel-related actions of COCA [22,25].

COCA overdose cases have recently been increasing in number [39,40]. In these cases, too, it is difficult to separately evaluate the relative contributions of the CNS-related vs. the peripheral mechanisms responsible for fatal COCA-induced toxicity, including CV toxicity. Some of the fatal, toxic symptoms caused by COCA that are not protected by CV drugs have been reported to be protected by some anticonvulsants (ACs) in humans [11,46] as well as in animals [43]. Furthermore, some of the seemingly CNS-related cardiotoxic effects of COCA have been reported to be ameliorated by suppressing COCA-induced seizures [21,23]. Therefore, COCA-induced cardiotoxic and convulsive effects seem to complement each other in causing fatal toxicity, and thus the elimination of both of these toxic effects should be the target of clinical treatment.

Interestingly, ethanol (EtOH), the most frequently coabused drug with COCA, has been reported to attenuate the severity of COCA-induced seizures while exacerbating the other toxic symptoms [31,33]. In particular, there is a characteristic enhancement of COCA-induced cardiotoxicity caused by EtOH that consequently exacerbates the net fatal toxicity [17,45]. On the other hand, the

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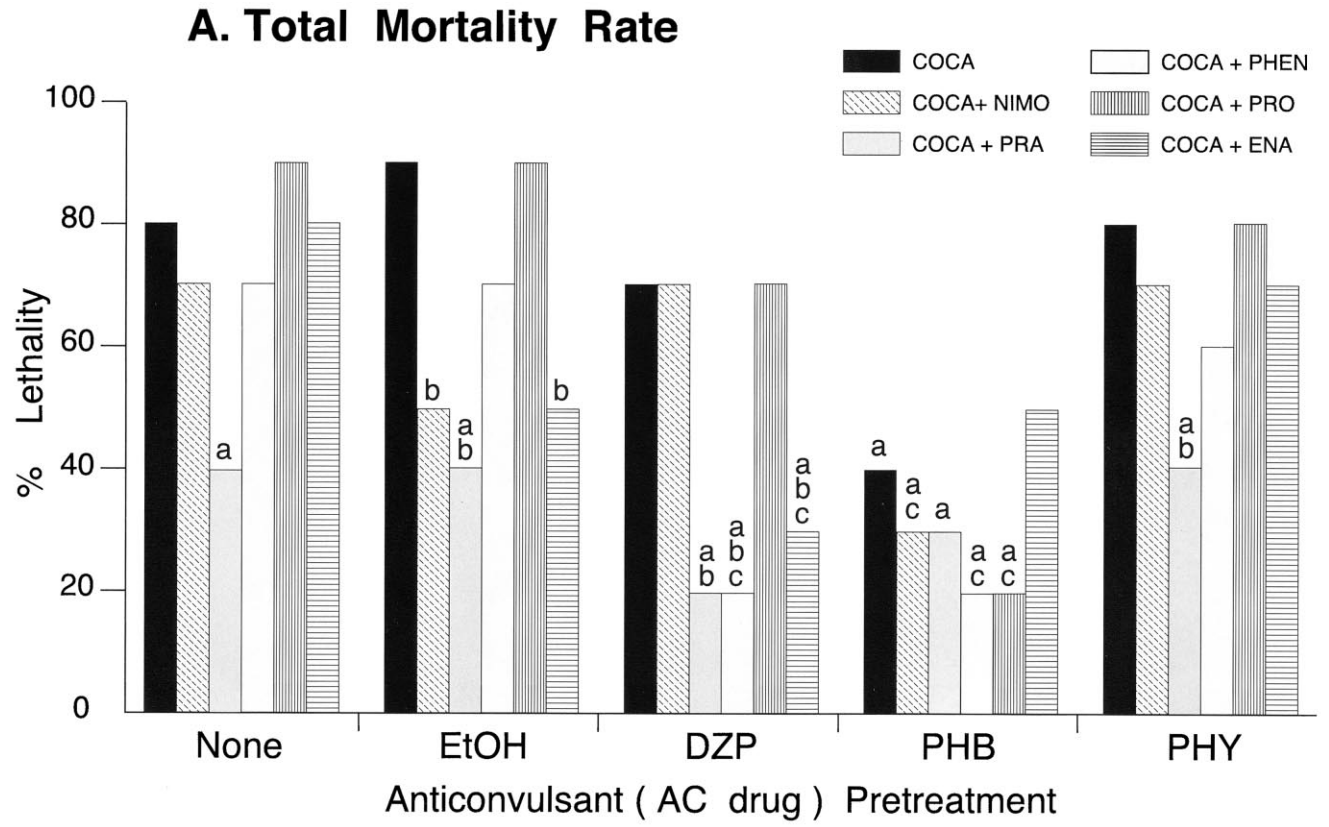
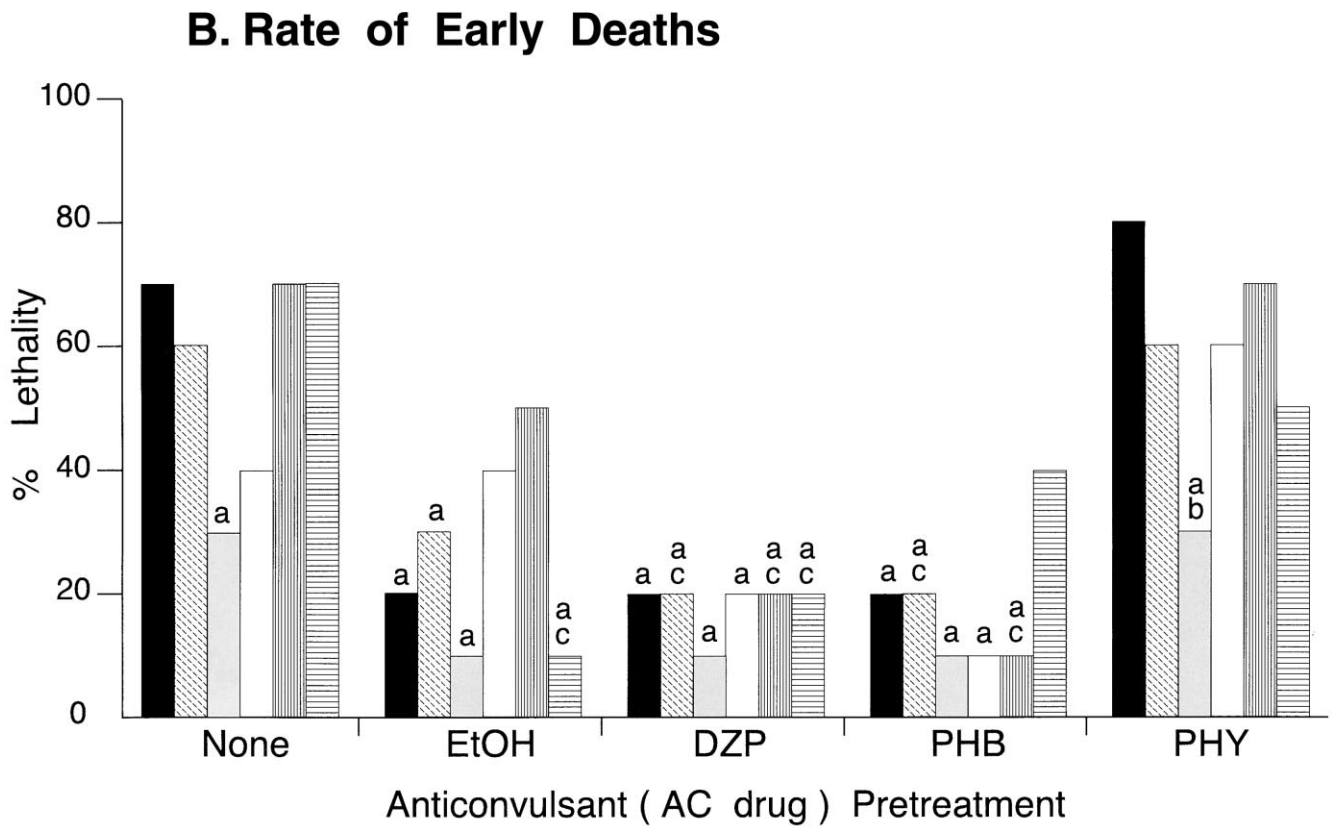

A. Total Mortality Rate
COCA
COCA+ NIMO
COCA + PRA
COCA + PHEN
COCA + PRO
COCA + ENA
% Lethality
100
80
60
40
20
0
None
EtOH
DZP
PHB
PHY
Anticonvulsant (AC drug) Pretreatment


B. Rate of Early Deaths
% Lethality
100
80
60
40
20
0
None
EtOH
DZP
PHB
PHY
Anticonvulsant (AC drug) Pretreatment

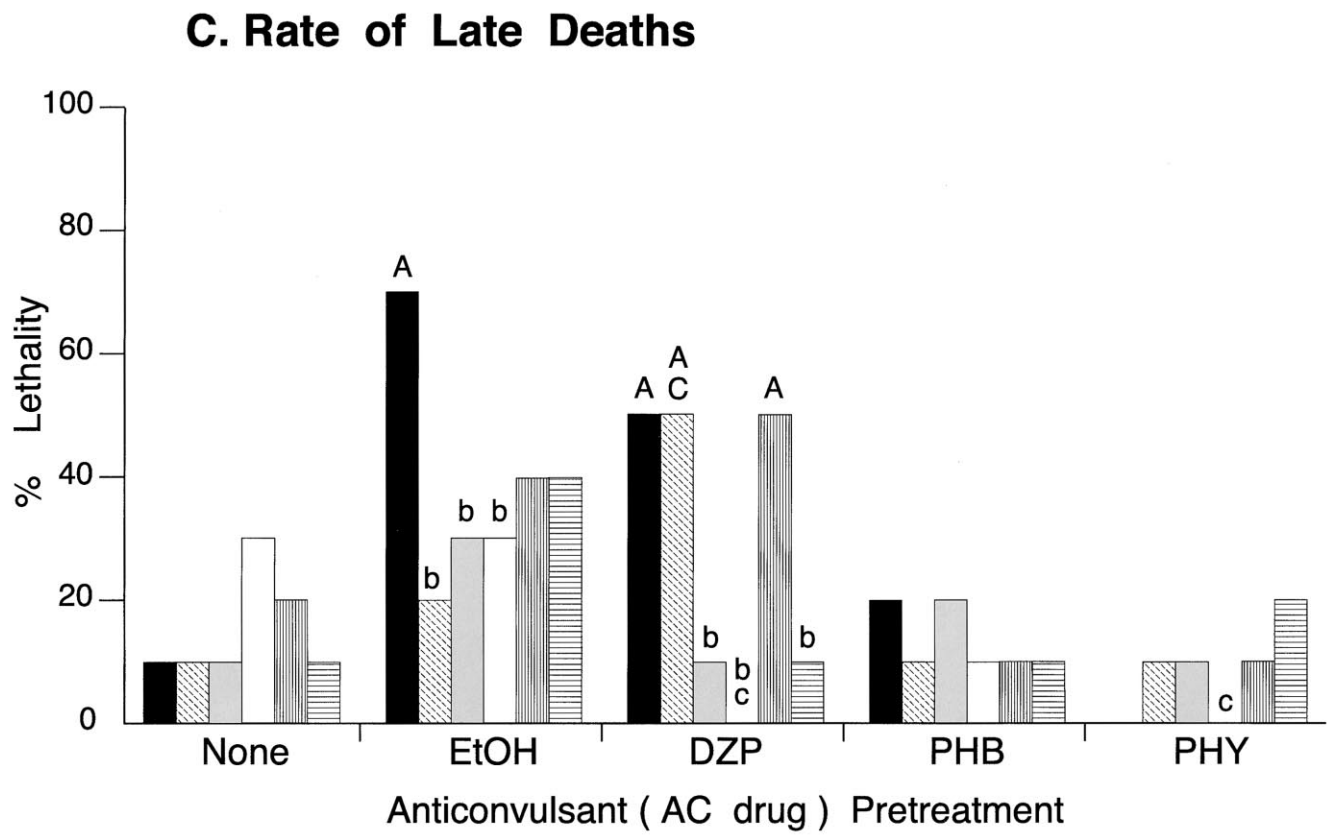


Fig. 1. Mortality rate (%) in the COCA groups treated with AC drugs and/or CV drugs in Experiment 1 ($n=10$). COCA, 75 mg/kg; EtOH, 3 g/kg; DZP, 5 mg/kg; PHB, 50 mg/kg; PHY 20 mg/kg; NIMO, 5 mg/kg; PRA, 5 mg/kg; PHEN, 5 mg/kg; PRO, 10 mg/kg; ENA, 10 mg/kg. In addition to the total mortality rate (A), the rates of the "early" and "late" deaths, which are defined in the text, are also demonstrated (B, C). The following differences were noted. (A, a) Significant ($p < 0.05$) increase (A) or attenuation (a) compared to the COCA-only group without AC or CV drug cotreatment. (B, b) Significant ($p < 0.05$) increase (B) or attenuation (b) compared to the corresponding COCA-AC drug-only group without CV drug cotreatment. (C, c) Significant ($p < 0.05$) increase (C) or attenuation (c) compared to the corresponding COCA-CV drug-only group without AC drug cotreatment.

combined use of a CV drug (such as propranolol, PRO) plus an AC (such as diazepam, DZP) has often become necessary in the emergency treatment of COCA toxicity cases [11,46] for the toxic symptoms described above. However, the actual therapeutic effects of these interventions have not been systematically investigated. Therefore, the purpose of this experiment was to investigate the enhanced antidotal effects against COCA toxicity, which could be caused by the combined use of CVs and ACs. Furthermore, by comparing the effects of EtOH and three representative ACs on the CV drug-modified toxic symptoms of COCA, the present study tried to elucidate some of the characteristics of the CNS-related and direct peripheral cardiotoxic effects of COCA.

2. Materials and methods

2.1. Animals and drug treatments

Male ICR mice (60–90 days old) were purchased from the Shizuoka Laboratory Animal Center (Hamamatsu, Japan), and were housed in a forced-air facility that was maintained at 23°C and 50% relative humidity, with a 12 L:12 D cycle [5]. The mice were kept in single cages (23.5 × 16.5 × 12 cm) with woodchip bedding, and were allowed water and lab chow ad lib. The experiments described in this report were conducted in accordance with the "Guidelines for Animal Experiments" of our institution [6], 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 the high dose of COCA the experiment was stopped and the mouse was euthanized. However, the COCA-induced convulsions and the related lethality were the subject of the present investigation.

Cocaine hydrochloride (COCA) (Takeda Chemical Industries, Osaka, Japan) was dissolved in saline, 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 causing a total mortality rate exceeding 50% in a preliminary experiment using the above breeding conditions, and with which

obvious convulsive and cardiotoxic effects have been previously reported [12,42]. However, high doses, against which previously reported antidotes had no effect [10,37], were avoided. The COCA was given 5 min after an IP injection of the CV drugs, which were administered with or without an IP injection of 3 g/kg EtOH (Nacalai Tesque, Kyoto, Japan) or the ACs, in a total volume of 10 ml/kg, dissolved in or diluted with saline. The doses for the CV or AC drugs were selected from the doses previously tested, which influenced some of the effects of COCA [9,10,42,43]. High drug doses, which by themselves caused any toxic effects like cardiac or respiratory dysfunction, were not used. The following CV drugs were used at nontoxic doses: nimodipine (NIMO) (2, 5, and 10 mg/kg) as a calcium channel blocker (Research Biochemicals, Natick, MA), prazosin (PRA) (2, 5, and 10 mg/kg) (Research Biochemicals) and phentolamine (PHEN) (2, 5, and 10 mg/kg) (Ciba-Geigy, Takarazuka, Japan) as α-adrenergic blocking agents (α1-selective and nonselective, respectively), PRO (2, 5, and 10 mg/kg) as a β-adrenergic blocking agent (Research Biochemicals), and enalapril (ENA) (5, 10, and 20 mg/kg) as an angiotensin converting-enzyme inhibitor (Sigma, St. Louis, MO). These CV drugs were initially studied in preliminary experiments. As AC drugs, DZP (1, 2.5, and 5 mg/kg) (Yamanouchi Pharmaceutical, Tokyo, Japan), phenobarbital (PHB) (50, 75, and 100 mg/kg) (Sankyo, Tokyo, Japan), and phenytoin (PHY) (10, 20, and 40 mg/kg) (Dainippon Pharmaceutical, Osaka, Japan) were initially studied. The most effective doses against the fatal toxic symptoms were then selected. When no significant amelioration of the fatal toxic symptoms was caused by any single dose of a CV or AC drug, then the highest nontoxic dose that did not enhance any toxic symptoms caused by 75 mg/kg COCA was selected. For the dose of EtOH, a nonlethal dose within the range in which its effects as an AC against the COCA-induced seizures were obvious (3 g/kg) was selected. For the other drugs, the final doses used were as follows: 5 mg/kg NIMO, 5 mg/kg PRA, 5 mg/kg PHEN, 10 mg/kg PRO, and 10 mg/kg ENA for the CV drugs, and 5 mg/kg DZP, 50 mg/kg PHB, and 20 mg/kg PHY for the AC drugs. Because NIMO could not be dissolved in saline, the solution for IP injection was obtained by dissolving it in a small amount of methanol (less than 5% of the total volume) that did not cause any significant behavioral alterations by itself, and then diluting the methanol solution with saline.

2.2. Evaluation of the toxic symptoms (Experiment 1)

With respect to the toxic symptoms, the CV drug-induced alterations in the mortality rate and seizures following an acute, single dose of 75 mg/kg COCA, with or without cotreatment with the AC drugs including EtOH, were examined in each group (n=10) (Figs. 1 and 2).

For the mortality rate, the fatality groups were classified based on a number of discriminatory variables (survival time, presence or absence of a temporary recovery of the early severe symptoms, presence of blood COCA) as previously reported [14]. Hence, the "early deaths", which were caused by the immediate and direct CV- and/or CNS-related effects of COCA, and the other delayed "late deaths" with a temporary recovery could be differentiated. In the present experiments, all of the "late-death" animals died more than 5 h after any of the "early-death" animals, and no COCA could be detected in the blood or brain of the "late-death" mice using the GC–EI–MS method described below.

The severity of the seizures was scored according to the methods described in a previous study [32]. A score of 0 (absence of convulsions), a score of 1 (short-lasting episodes of clonic convulsions), a score of 2 (clonic convulsions with loss of the righting reflex), a score of 3 (episodic convulsions including convulsions lasting longer than 5 min), and a score of 4 (episodic convulsions continuous and violent enough to cause death) could be differentiated.

2.3. Examination of COCA levels (Experiment 2)

Considering the modified COCA metabolism caused by the acute COCA–CV–AC drug interactions, the COCA concentrations in the blood and brain at 5 min after the COCA administration were evaluated for the other groups of mice treated with the same protocols (n=4). No mice had died before that time point. After the mice were sacrificed at 5 min, the blood samples were collected in 0.5% sodium fluoride, and the brain samples were rinsed with ice-cold 0.9% saline. All of the samples were homogenized in 8 volumes of 5% trichloroacetic acid, and COCA was extracted from the supernatants using Bond Elut Certify LRC solid-phase columns (Analytichem International, Harbor City, CA) [7,14], with the synthesized internal standard propylbenzoylecgonine [18]. The COCA levels were determined by a GC–EI–MS method, using a Shimadzu QP-2000A connected to a Shimadzu GC-14A gas chromatograph with a CBP5-M25-025 column (the analytical instruments and the column were provided by Shimadzu, Kyoto, Japan) and helium carrier gas [13,14]. The oven temperature was programmed from 90°C to 280°C at 20°C/min, with an initial hold time of 2 min and a final hold time of 3 min. The injection port and ion source temperatures were both 250°C, and the ionization energy was 70 eV. Quantitative analysis of COCA was performed by estimating the major mass ions (m/z 182 and 303) in the samples vs. the standards (1, 5, 10, 20, or 50 μg/ml or μg/g), which were prepared from blank blood and brain homogenates [18].

2.4. Data analysis

After the data were subjected to one-way analysis of variance (ANOVA), the chi-square test was used to

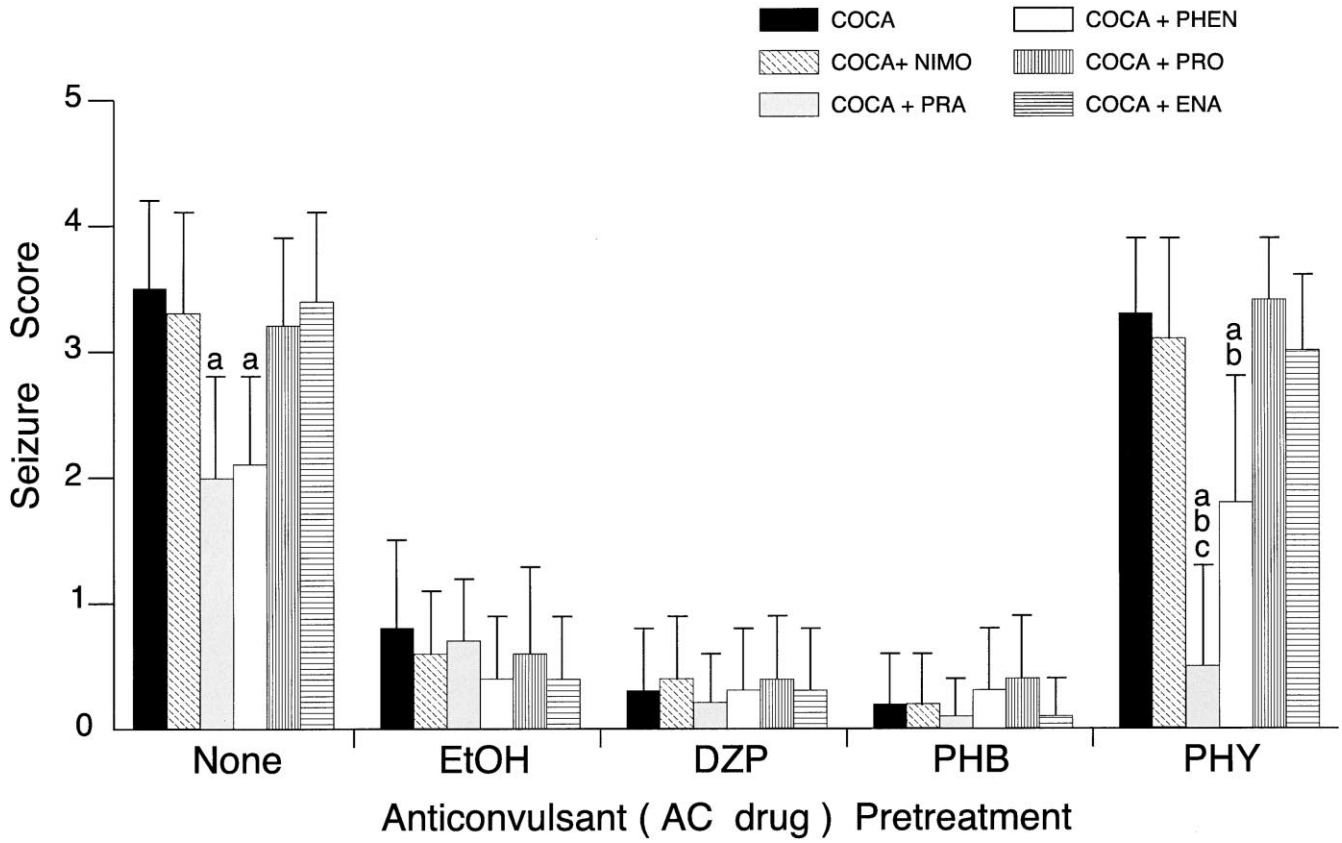


Fig. 2. Seizure score in the COCA groups treated with AC and/or CV drugs in Experiment 1 ($n=10$). All of the abbreviations used are the same as in Fig. 1. The data represent means ± SD. In the EtOH-, DZP-, and PHB-cotreated groups all of the seizure scores were significantly ($p<0.05$) lower than in the COCA-only and the corresponding COCA-CV drug-only groups without AC drug cotreatment (not shown in this figure). The other differences were noted in the same way as in Fig. 1.

compare the percent lethality (ratio of the number of dead mice out of all mice used) between groups, and a two-sample *t*-test with Welch's correction was used to compare the seizure scores and the drug levels. All of the comparisons were performed using a statistical software package (Shakai Johou Service, Tokyo, Japan) and its manual [36]. Unless otherwise noted, *p*-values less than 0.05 were considered to be statistically significant.

3. Results

3.1. Alteration of the mortality rate in Experiment 1 (Fig. 1)

After comparing the effects of each AC drug without CV drug cotreatment, we found that only PHB protected against the overall COCA-induced lethality (Fig. 1A, black bars). The total mortality rate was not attenuated in either the EtOH-, DZP-, or PHY-treated group (Fig. 1A). In particular, the rate of late deaths was increased in the EtOH- and DZP-treated groups (Fig. 1C), whereas mainly early deaths were observed in the PHY-treated groups (Fig. 1B).

Among the CV drugs used in a single treatment (the six leftmost bars in Fig. 1), only PRA, an α1-adrenergic blocking agent, significantly attenuated the mortality rate.

In the EtOH-treated groups, the total mortality rate was significantly attenuated by the calcium channel blocker NIMO and the angiotensin converting-enzyme inhibitor ENA, as well as by PRA, compared to the COCA–EtOH-only group (Fig. 1A). However, only in the COCA–EtOH group cotreated with PRA the total mortality rate was lower than in the COCA-only group (Fig. 1A).

In the DZP-treated groups, protective effects caused by PRA, PHEN, and ENA were observed, compared to both the COCA–DZP-only and the COCA-only groups (Fig. 1A). Similar to the EtOH-treated groups, the attenuation in the mortality rate was observed mainly in the late death groups (Fig. 1C).

In the PHB-treated groups, the protective effects of PHB against the lethality were preserved in most of the CV drug-cotreated groups. In the ENA-cotreated group, however, a slight increase in the total mortality rate was observed as compared to the COCA–PHB-only group (Fig. 1A).

Table 1
Early COCA concentrations in Experiment 2

	COCA	COCA + NIMO	COCA + PRA	COCA + PHEN	COCA + PRO	COCA + ENA
(A) Non-AC drug groups						
Blood	19.24 ± 1.77	15.88 ± 1.34[a]	14.25 ± 1.31[a]	14.10 ± 1.25[a]	15.05 ± 1.21[a]	14.35 ± 1.22[a]
Brain	43.15 ± 3.21	42.71 ± 3.56	33.64 ± 3.00[a]	35.83 ± 3.13[a]	43.33 ± 3.26	39.39 ± 3.78
(B) EtOH-cotreated groups						
Blood	13.42 ± 1.58[a]	14.43 ± 2.01[a]	11.52 ± 1.47[a,c]	15.28 ± 2.16[a]	14.47 ± 1.93[a]	13.45 ± 1.39[a]
Brain	42.23 ± 3.05	47.67 ± 4.22[B]	40.35 ± 2.79[C]	47.86 ± 4.36[B,C]	50.02 ± 4.79[A,B,C]	42.50 ± 2.92
(C) DZP-cotreated groups						
Blood	14.35 ± 1.85[a]	12.97 ± 1.85[a,c]	10.80 ± 1.40[a,c]	14.15 ± 2.25[a]	12.87 ± 2.00[a]	11.62 ± 1.73[a,b,c]
Brain	34.24 ± 2.65[a]	43.26 ± 3.71[B]	39.23 ± 2.58[B,C]	43.17 ± 4.22[B,C]	47.25 ± 3.98[B]	40.34 ± 3.51[B]
(D) PHB-cotreated groups						
Blood	12.01 ± 1.26[a]	13.43 ± 1.37[a,c]	13.27 ± 1.78[a]	12.47 ± 1.33[a]	13.43 ± 2.02[a]	14.06 ± 2.33[a]
Brain	32.32 ± 2.46[a]	32.14 ± 2.39[a,c]	31.36 ± 2.53[a]	32.14 ± 2.54[a]	34.65 ± 3.01[a,c]	36.23 ± 3.20[a]
(E) PHY-cotreated groups						
Blood	19.11 ± 2.75	16.47 ± 2.31	15.48 ± 2.13[a,b]	14.43 ± 2.11[a,b]	18.05 ± 2.64	16.34 ± 2.26[a]
Brain	41.37 ± 3.43	41.48 ± 3.67	40.96 ± 3.21[C]	39.43 ± 3.49	45.34 ± 4.11	42.35 ± 3.96

Early COCA concentrations in the blood and brain samples in Experiment 2 ($n=4$). The COCA concentrations at 5 min after the drug administration, using the same protocol as in Experiment 1, are demonstrated. All of the abbreviations used are the same as in Fig. 1. The data represent means ± SD, and the differences were noted in the same way as in Experiment 1.

In the PHY-treated groups, the protective effects of PRA against lethality were preserved.

3.2. Alteration of the seizures in Experiment 1 (Fig. 2)

In a single treatment, EtOH, DZP, and PHB protected against the COCA-induced seizures. PHY 20 mg/kg, like the other test doses above (10 and 40 mg/kg), which enhanced the COCA-induced toxic symptoms in preliminary trials failed to protect against either the seizures or the lethality. Among the CV drugs used, PRA and PHEN suppressed the COCA-induced seizures.

The AC effects of EtOH, DZP, and PHB were unaffected by cotreatment with the CV drugs. In the PHY-treated groups, the AC effects of PRA and PHEN were preserved.

3.3. Alteration of COCA levels in Experiment 2 (Table 1)

The coadministration of the CV and/or AC drugs with COCA at a short interval (5 min) caused acute, significant alterations in the blood and/or brain COCA levels at 5 min after the administration (Table 1). However, the decrease in the COCA concentration of the blood and/or brain was not always accompanied by the attenuation of the mortality rate observed in Experiment 1. Nevertheless, the attenuation of severe seizures in a single PRA-, PHEN-, DZP-, or PHB-cotreated group was accompanied by a decrease in the brain COCA concentration. In the COCA–PHB groups, a significant decrease in both blood and brain COCA levels was observed, but the most moderate decrease in the COCA levels was observed in the group cotreated with ENA with no decreased mortality rate (Table 1D).

4. Discussion

Because both COCA-induced seizures and CV toxicity are closely correlated with COCA-induced lethality, the effects of AC and CV drugs have been separately examined previously [2,9,41,42]. However, their interactions have not been systematically investigated. The effects of CV drugs, especially their AC effects, are controversial depending on the circumstances of the fatal COCA-induced toxicity [2,8,41–43]. Nevertheless, in the present experiment employing an acute COCA administration combined with nontoxic doses of each CV drug, the protective effects on the COCA-induced seizures were demonstrated for the α-adrenergic blocking agents. Both α_1- and α_2-adrenergic antagonisms have been reported to be correlated with the suppression of COCA-induced seizures [41]. However, the absence of any significant protective effects of PHEN against COCA-induced lethality (Fig. 1) seemed to support an enhancement of the reflex cardiostimulating effects of α_2-adrenergic blocking agents with exacerbated ischemic cardiac events [44]. Pure antagonism of the α_1-adrenergic system, on the other hand, has been reported to ameliorate the acute hypoxia and acidosis accompanying COCA-induced cardiotoxicity [43].

By combining the AC drugs with the CV drugs, strong antidotal effects against COCA toxicity were often observed. Furthermore, by differentiating the early deaths from the late deaths, which were increased in the groups cotreated with EtOH or DZP, some characteristics of the EtOH- and DZP-CV drug interaction were elucidated. Although an enhancement of COCA toxicity by EtOH has been frequently reported, the calcium channel blocker NIMO seemed to be effective against the slow but continuous cardiotoxicity enhanced by EtOH, which has been reported to be related to a pathological constriction of the blood vessels [17,45]. It could also be postulated that the α_1-adrenergic blocking agent PRA provided similar prolonged protective effects, in addition to its immediate effects against the COCA-induced seizures and other early toxic symptoms. Furthermore, differences in the nature of the late deaths between the EtOH-cotreated and the DZP-cotreated groups were suggested, although the toxic symptoms could not be visually differentiated. One of the obvious differences between EtOH and DZP was their effect on the COCA–NIMO interaction. In the COCA–DZP group, unlike in the COCA–EtOH group, the rate of late deaths was not attenuated by NIMO. NIMO, when combined with DZP, has been reported to cause toxic vasodilation in the brain [24], whereas the deleterious CV effects of EtOH have been reported to be ameliorated by NIMO [29]. Therefore, when ENA, an angiotensin converting-enzyme inhibitor effective only on limited distal vessels [47], was combined with DZP, an enhanced protective action stronger than either DZP or ENA alone was provided. Enhanced protective effects were also observed for the DZP–PHEN combination, in which PHEN has been reported to block the unfavorable effects of DZP via the actions of α_2-adrenergic receptors [28].

Of the AC drugs examined in the present study, strong antidotal effects were noted for PHB, with or without the CV drug cotreatment. These effects of PHB, which likely potentiates synaptic inhibition through actions on GABAA (γ-aminobutyric acid) receptors [30], were different from another GABA receptor-related drug DZP, which enhances the GABA-induced increase in the conductance of CI^- as a benzodiazepine receptor agonist [30]. Following treatment with PHB, the mortality rate including the rate of late deaths was decreased, even in the group of mice cotreated with PRO, which did not ameliorate the COCA toxicity in any other AC drug combination. Some favorable CV effects seemingly related to β-adrenergic receptors have been reported for PHB [1,3], although the precise mechanisms have not been elucidated. The long-lasting effects of PHB may be due to its longer half-life compared to DZP [30].

The alteration in COCA metabolism may also be related to the strong protective effects of PHB, considering the results in Table 1, which demonstrated the decreased COCA levels in the blood and brain samples of all the groups cotreated with PHB (Table 1D). In the PHB-ENA group, in which the decrease in the COCA levels was the most moderate in the PHB-treated groups, the mortality rate was not significantly attenuated. However, the decreased COCA levels observed in the blood and/or brain samples of mice in the other administration groups were not always accompanied by a decreased seizure score or by a decreased mortality rate. For example, despite the decreased COCA levels in both the blood and brain, and the decreased seizure score, the mortality rate was not attenuated in the group cotreated with a single dose of PHEN or DZP. Furthermore, in the EtOH-treated groups, the AC effects were not accompanied by decreased brain COCA levels. Even in the PHB-treated groups, detailed examinations of the alterations in COCA metabolism that focus on the early vs. late COCA distributions, as well as an examination of the unfavorable PHB–ENA interactions, which have not been elucidated, will be required. However, the AC effects of the other AC drugs, especially EtOH, seemed to be more independent of COCA metabolism than the effects of PHB, even if the profile and strength of the AC effects resembled those of PHB [34]. Despite a decreased rate of COCA elimination [15,16], accompanied by increased COCA levels (Table 1), strong AC effects (Fig. 2) were provided by a high dose (3 g/kg) of EtOH. These AC effects were seemingly due to its strong effects on GABA and other seizure-related CNS receptors (ex. N-methyl-D-aspartate (NMDA) receptors), which have been reported previously [26]. The AC effects of DZP have also been reported to be independent of COCA metabolism [4], although decreased COCA levels were observed in some DZP-treated groups in the present study (Table 1C).

Apart from COCA metabolism, the mechanisms related to the effects of PHB have been reported to be different from the mechanisms responsible for the actions of DZP or EtOH [27]. PHB seemed to antagonize the COCA effects more strongly than DZP on peripheral and CNS receptors correlated with the COCA-induced seizures and cardiotoxicity (ex. NMDA and adrenergic receptors) [1,3,8,10,27]. Furthermore, the direct cardiotoxic effects of PHB [19] are weaker than those of EtOH, which exacerbates the cardiotoxic effects of COCA directly or by synthesizing cardiotoxic metabolites such as cocaethylene [15–17,45]. Unlike the GABA receptor-related drugs (EtOH, DZP, and PHB), PHY, which does not act on any specific receptors, was ineffective against the COCA-induced seizures and lethality. PHY prolongs the refractory inactivation period of voltage-activated Na^+ channels, and thus reduces the ability of neurons to fire at high frequencies [30]. The decrease of brain COCA levels was not observed, either, even when the PHY dose was altered (10 or 40 mg/kg) (data not shown). Therefore, both COCA metabolism and some modifications of GABAergic neurotransmissions or other seizure-related receptors seemed to be correlated with the antidotal effects against COCA-induced seizures and lethality.

It can be concluded that the net antidotal effects were enhanced or preserved by combining PHB with NIMO, PRA, PHEN, or PRO, as well as by combining DZP with PRA, PHEN, or ENA. Even when EtOH was combined with COCA, antidotal effects of PRA were preserved. It can also be concluded that the COCA-induced seizures were closely correlated with GABA receptor-related mechanisms in addition to α-adrenergic mechanisms.

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