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Brain β-Endorphin Immunoreactivity as an Index of Cocaine and Combined Cocaine–Ethanol Toxicities

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HAYASE, T., H. ABIRU, Y. YAMAMOTO, K. YAMAMOTO AND Y. FUKUI. *Brain β-endorphin immunoreactivity as an index of cocaine and combined cocaine–ethanol toxicities*. PHARMACOL BIOCHEM BEHAV. **60**(1) 263–270, 1998.—The present study examines alterations in the cytoplasmic immunoreactivity of brain β-endorphin, an endogenous opioid peptide regarded as the mediator of both euphoria and antinociceptive systems, in relation to toxicities due to cocaine and combined cocaine–ethanol. β-endorphin–immunoreactive cells were visualized and counted in adjacent sections from male rat brains at the level of the arcuate nucleus. In this region, cytoplasmic β-endorphin immunoreactivity is prevalent. An intraperitoneal injection of cocaine (75 or 15 mg/kg) was given 15 min after an intraperitoneal injection of 3 g/kg ethanol or vehicle. With a fatally toxic dose (75 mg/kg) of cocaine, the number of neurons exhibiting cytoplasmic β-endorphin immunoreactivity (immunoreactive nerve cells) was significantly increased immediately after the drug administration. Ethanol further enhanced the effects of both 15 and 75 mg/kg of cocaine. When the immunoreactivity was visually estimated by computer imaging analysis, lightly stained, weakly immunoreactive cells with photographic light absorption values greater than 50% were enhanced in the cocaine–ethanol groups compared to the cocaine only groups. Fatal toxicities were only observed in the groups treated with the high cocaine doses (75 mg/kg), with or without ethanol. In these groups, the number of strongly immunoreactive cells had increased significantly compared to the other groups. In the group treated with the high cocaine dose (75 mg/kg) plus ethanol, an increased frequency of late deaths that occurred over 1 h after the drug administration was observed, together with a decreased severity of cocaine-induced seizures and an early enhancement of weakly immunoreactive cells. Unlike the strongly immunoreactive cells, the weakly immunoreactive cells appeared to be continuously enhanced, based on an experiment examining β-endorphin immunoreactivity at 24 h after an injection of 50 mg/kg cocaine. © 1998 Elsevier Science Inc.

Cocaine Ethanol β-Endorphin Immunoreactivity Toxicity Fatality Seizures

DEATHS caused by an overdose of cocaine or combined cocaine–ethanol have recently become more frequent with the increased usage of these drugs (25). With respect to the human fatalities caused by these drugs, the possible mechanisms underlying their lethal effects have been discussed (11,29). By labeling those brain sites suspected to be related to these effects and then examining the binding potencies of these drugs in intoxicated human autopsy cases, the sites which seem to be correlated with the lethal effects, for example, the dopamine transporter, can be specified. However, satisfactory indices for evaluating these fatal toxicities quantitatively have not been elucidated. Although previous studies have suggested that the drug concentrations at the time of death can be used as indices for fatal toxicity, delayed drug-induced deaths have occurred in both humans (11,13) and mice (9), even though no drugs were detected at the time of death. Delayed deaths from overdose generally involved use of cocaine or combined cocaine–ethanol.

β-Endorphin is an endogenous opioid peptide that is released in response to a number of physical stresses, and is regarded as the mediator of both euphoria and antinociceptive systems (12,22). The plasma levels of β-endorphin have been reported to be continuously increased during cocaine abuse, but continuously decreased during ethanol abuse in humans (33,34). A recent study in humans that collected cases of acute ethanol intoxication demonstrated increased levels of plasma

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β-endorphin in drinkers and decreased levels in nondrinkers (1). Elevated levels of β-endorphin are also observed in convulsions like those observed in human cocaine toxicity (24). In rats, elevated levels of β-endorphin are also reported in fatal respiratory disorders (31). Although the conditions under which these case studies were examined were different, when taken together, the possibility of a relationship between fatal cocaine or cocaine-ethanol toxicity and β-endorphin levels is suggested. Direct effects of cocaine and ethanol on the endogenous opiate system have also been previously demonstrated (3,5,8). Furthermore, a relationship between cocaine or combined cocaine–ethanol toxicity and brain opioid receptors is supported by the fact that buprenorphine, a synthetic ligand for opioid receptor, can protect against their toxicities in mice (10). Other previous studies in rats have demonstrated that fatal toxicities due to cocaine administrations were also modified by more than one opioid receptor ligand (6). Furthermore, β-endorphin levels, including plasma β-endorphin levels, are subject to the opioid receptors themselves and are influenced by both agonists and antagonists of each receptor subtype, including buprenorphine, in humans (17). Therefore, the above effects of buprenorphine against fatal cocaine or combined cocaine–ethanol toxicity seems to support a correlation between these fatal toxic cases and β-endorphin levels. This study attempted to elucidate the relationship between the toxic effects of cocaine or combined cocaine–ethanol and changes in the β-endorphin levels of rat brain sections. Although there are fewer studies on the effects of these drugs on the brain β-endorphin immunoreactivity than on the plasma levels of β-endorphin, a limited number of experiments have demonstrated some direct and immediate effects on the brain β-endorphin immunoreactivity in rats (2,30). Furthermore, based on the previously reported differences in the time of death caused by cocaine and cocaine–ethanol (9,10,28), we tried to classify the types of fatal toxicities, observing behavioral disorders (respiratory disorders, locomotive disturbances, and seizures) reported in mice (19). Then we focussed on the β-endorphin immunoreactivity in delayed intoxication, in which the drugs were already at nondetectable levels in the body. After all, three types of experiments were performed to investigate the relationship between early and late deaths and the immunocytochemically demonstrated brain β-endorphin levels.

METHOD

Animals and Drugs

Adult male Wistar rats (290 ± 60 g) were purchased from the Shizuoka Laboratory Animal Center, and were maintained under the conditions previously reported (20) in single cages with a 12-L:12-D cycle. All drug administrations were performed between 1930 and 2130 h. The experiments described in this report were conducted in accordance with the Guideline for Animal Experiments of Kyoto University, as drafted in 1988, which is founded on the National Institutes of Health Guide for Care and Use of Laboratory Animals. Cocaine hydrochloride was provided by Takeda Chemical Industries Ltd. Pentobarbital sodium (Nembutal) was provided by Dainippon Pharmaceutical Industries Ltd. All other chemicals used were of guaranteed reagent grade, and were obtained from Nacalai Tesque Co. Ltd.

Experimental Protocols

In Experiment 1, an intraperitoneal injection of cocaine (75 or 15 mg/kg) was given 15 min after an intraperitoneal injection of saline (cocaine groups: groups B and C) or 3 g/kg ethanol (cocaine–ethanol groups; groups E and F) (Fig. 1. n = 4 for each group). Ethanol was diluted in saline and was administered in a final volume of 10.0 ml/kg. Cocaine hydrochloride was dissolved in saline and was administered in a final volume of 5.0 ml/kg. In the control group without any drug administration (group A), the same volumes of saline per kilogram body weight were administered: 5.0 ml/kg saline was given 15 min after 10.0 ml/kg saline injection. In the control group without cocaine (group D), 5.0 ml/kg saline was given 15 min after a 3 g/kg ethanol injection, under anesthesia induced by an intraperitoneal injection of 60 mg/kg pentobarbital (21).

Intracardial perfusion was initiated 3 min after the cocaine injections to examine the early immunoreactivity before any of the animals could die. The immunocytochemical studies were carried out on adjacent sections at each level of the arcuate nucleus, where cytoplasmic β-endorphin immunoreactivity is prevalent in normal male rats (18,20). After the rats were perfused intracardially with 300 ml of phosphate-buffered saline (PBS) containing 4% formaldehyde (pH 7.4), the brains were removed and postfixed in PBS containing 4% formaldehyde and 15% sucrose (pH 7.4). The brains were then sectioned coronally at representative levels of the arcuate nucleus. Considering the regional differences in the architecture and function of the arcuate nucleus previously reported (4,20), adjacent sections were obtained from two levels: a more rostral (between bregma −2.0 and −2.8 mm) level, and a more caudal (between bregma −2.8 and −3.8 mm) level, in reference to the drawings from the textbook (23). Three sections per level were used for each group to examine the immunoreactivity. The immunocytochemistry was performed with a commercial immunofluorescence kit using the FITC (fluorescein-isothiocyanate) method (Peninsula Laboratories, Inc.). Following the instructions accompanying the kit (7), the FITC-conjugated goat antirabbit gamma globulin was applied to each section as the secondary antibody, after the application of the primary antibody. Observation of the stained immunoreactive neurons was performed with a dark-field microscope (Nikon Fluophot connected to a FX-35 camera) for the detection of incident light fluorescence. The number of neurons exhibiting cytoplasmic immunoreactivity (immunoreactive nerve cells) was counted per one field of vision at a magnification of 400×. The cell counts were performed in four different fields in the periventricular area. Black-and-white photomicrographs of the immunoreactive nerve cells in each field obtained by the above apparatus were scanned using a computer software package (Adobe Photoshop, 3.0J, Adobe Systems Inc.), and the strength of the immunoreactivity in focus was visually differentiated by estimating the light absorption values (%), with the most luminous part regarded as 0%. False immunopositive reactions were excluded according to the instructions accompanying the kit (7). After comparing the β-endorphin immunoreactivity between each level within each group, comparisons between the groups were performed.

In Experiment 2, after confirming that fatal toxicities only occurred with the protocols using 75 mg/kg cocaine, two other groups of rats treated with these protocols (groups C′ and F′ of Table 2; n = 7 for each group) were also examined continuously for various observable disorders accompanying the fatal toxicities. These disorders were scored depending on the severity in both surviving and dead animals, and mean values were calculated for each group. We also attempted to classify the fatalities to differentiate the late fatalities previously demonstrated in mice, in which no drugs were detected at the time

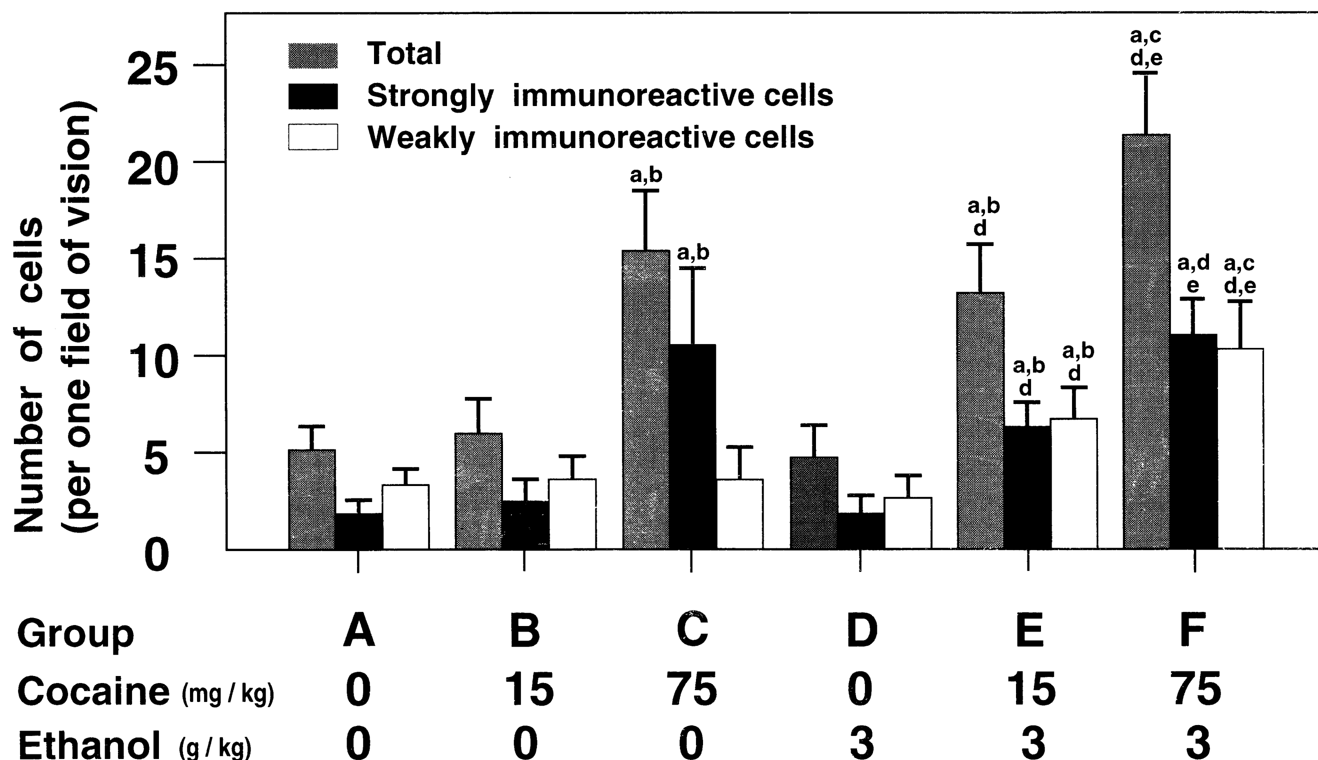

FIG. 1. Early immunoreactivity at 3 min after cocaine administration in Experiment 1 (mean ± SD). Weakly immunoreactive cells with light absorption values greater than 50% (more blackish) and strongly immunoreactive cells with the light absorption values less than 50% (more whitish) were calculated differently, and were averaged as defined and explained in the text. Significant increases in the number of corresponding cells were defined as follows: a: Significant increase compared to Group A. b: Significant increase compared to Group B. c: Significant increase compared to Group C. d: Significant increase compared to Group D. e: Significant increase compared to Group E.

of death, from earlier fatalities by means of the observed disorders as well as the survival times (9,10,28). A preliminary study over a 1-week continuous observation period demonstrated that no rats had died over 24 h after the cocaine injection, when it was performed properly. Therefore, a continuous 24-h observation period was performed in this study and rats other than those that died within 24 h after the injection were regarded as survivors.

In Experiment 3, an intraperitoneal injection of cocaine (50 mg/kg) was given 15 min after an intraperitoneal injection of vehicle (group H of Fig. 2) or 3 g/kg ethanol (group J of Fig. 2), as per the other groups of rats ($n = 4$ for each group). The injection volumes and the preparation of the control group (group G) are the same as in Experiment 1. However, based on the preliminary study described above, the same intracardial perfusion and immunocytochemical studies as in Experiment 1 were performed 24 h after the drug administration. Strictly speaking, the survival time of each case of fatal toxicity seemed to be different. However, by performing the immunocytochemical studies 24 h after the drug administration, the tendencies of the late immunoreactivity as correlated with the late effects of these drugs could be determined. To avoid the occurrence of death due to toxicity, a lower dose of cocaine was chosen in this experiment than in Experiment 1.

Statistical Analysis

Throughout the present experiments, a two-sample t-test with Welch's correction was used to compare the mean values, and a chi-square test was used to compare the percentage of dead rats to the total number of rats used. The mean values included the mean number of cells in each group, as defined in the Results. P-values less than 0.05 were considered to be statistically significant.

RESULTS

Significant regional differences in the number of cells were not observed. Therefore, after the cell counts were performed on four different fields in the periventricular area, the average of these four values was calculated. Next, the average of the three slices, and then the average of the two levels of the arcuate nucleus was calculated. The value obtained in this way was defined as the mean number of cells in each group of this experiment.

In the cocaine-only groups of experiment 1, a toxic dose (75 mg/kg) significantly increased the total number of nerve cells exhibiting cytoplasmic immunoreactivity for β-endorphin (group C of Fig. 1), compared to the control group without cocaine (group A of Fig. 1). In contrast, the lower dose (15 mg/kg) did not cause any significant effect (group B of Fig. 1). In the cocaine-ethanol groups, ethanol further enhanced the effects of 75 mg/kg cocaine (group F of Fig. 1); the immunoreactivity caused by 75 mg/kg cocaine had increased significantly compared to the corresponding nonethanol group (group C of Fig. 1). Furthermore, with 3 g/kg of ethanol, even the immunoreactivity provided by 15 mg/kg of cocaine had increased significantly (group E of Fig. 1) compared

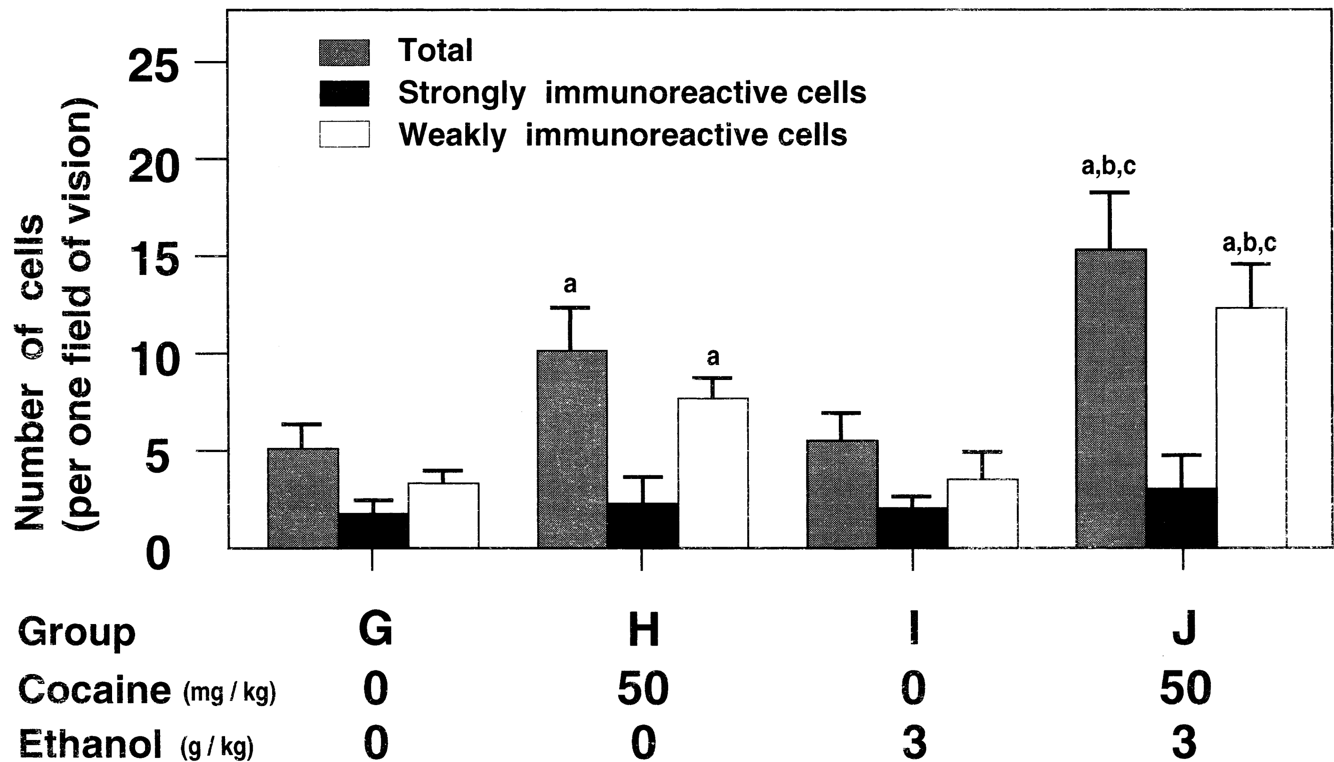

FIG. 2. Late immunoreactivity at 24 h after cocaine or cocaine–ethanol administration in Experiment 3. The animals were sacrificed 24 h after the administration, and cell counting was performed in the same manner as in Fig. 1. a: Significant increase compared to Group G. b: Significant increase compared to Group H. c: Significant increase compared to Group I.

to the corresponding nonethanol group (group B of Fig. 1). Computer image analysis revealed that the immunoreactive cells could be visually differentiated, depending on the strength of their immunoreactivity, as indicated by their light absorption values (%) (Fig. 3). In the present experiment, lightly stained immunoreactive nerve cells with light absorption values greater than 50% (more blackish) were defined as weakly immunoreactive cells, whereas more intensely stained immunoreactive nerve cells with light absorption values less than 50% (more whitish) were defined as strongly immunoreactive cells. This classification of immunoreactive cells revealed an enhancement of weakly immunoreactive cells in the cocaine–ethanol groups (groups E and F of Fig. 1), compared to the corresponding nonethanol groups (groups B and C of Fig. 1). The number of strongly immunoreactive cells also increased significantly in the 15 mg/kg cocaine plus 3 g/kg ethanol group (group E of Fig. 1), compared to the corresponding nonethanol group (group B of Fig. 1). However, this enhancement of strongly immunoreactive cells was much less when compared to the groups treated with 75 mg/kg cocaine (groups C and F of Fig. 1).

In Experiment 2, drug-induced disorders related to the fatal toxicities were examined using a protocol of 75 mg/kg cocaine with or without 3 g/kg ethanol (groups C′ and F′ following the drug administration protocol for groups C and F in Experiment 1, respectively), following which drug-induced deaths could be observed. The observable disorders were estimated under the criteria shown in Table 1, and a comparison was made between the cocaine group (group C′ of Table 2) and the cocaine–ethanol group (group F′ of Table 2). These disorders included respiratory disorders, locomotive disturbances, and seizures. For the respiratory disorders and locomotive disturbances, mild disorders that did not result in the death of the rat were regarded as score 0 disorders. Furthermore, insidious disorders observed mainly in the late fatalities, which were moderate or not obvious until near the time of death, were defined as score 1 disorders, and were differentiated from serious (immediate and mortal) disorders (score 2 disorders). The seizures were scored depending on the presence (scores 1 and 2) or absence (score 0) of the seizures themselves, and then depending on the presence (score 2) or absence (score 1) of the seizures with obvious tonic–clonic activity, regardless of their durations. Late fatalities previously reported in mice, in which no drugs were detected at the time of death (9,10,28), were also observed in the rats. They occurred at least over 7 h after the cocaine administration. Earlier fatalities could be clearly differentiated from these late fatalities, as they occurred within 20 min after the cocaine administration, and were accompanied by serious disorders. Considering the possibility of temporally exceptional deaths due to ethanol-induced fatalities in mice (15), all of the deaths that occurred within 1 h after the cocaine administration were defined as "early deaths," and all of the deaths that occurred over 1 h after the cocaine administration were defined as "late deaths" in Table 1. However, the early deaths could be clearly differentiated from the late deaths, as shown in Table 1, and no rats died during any other peiod. The frequency of late deaths increased in the cocaine–ethanol group (group F′ of Table 2) compared to the cocaine-only group (group C′ of Table 2). Using the protocol of group C′ (75 mg/kg cocaine plus

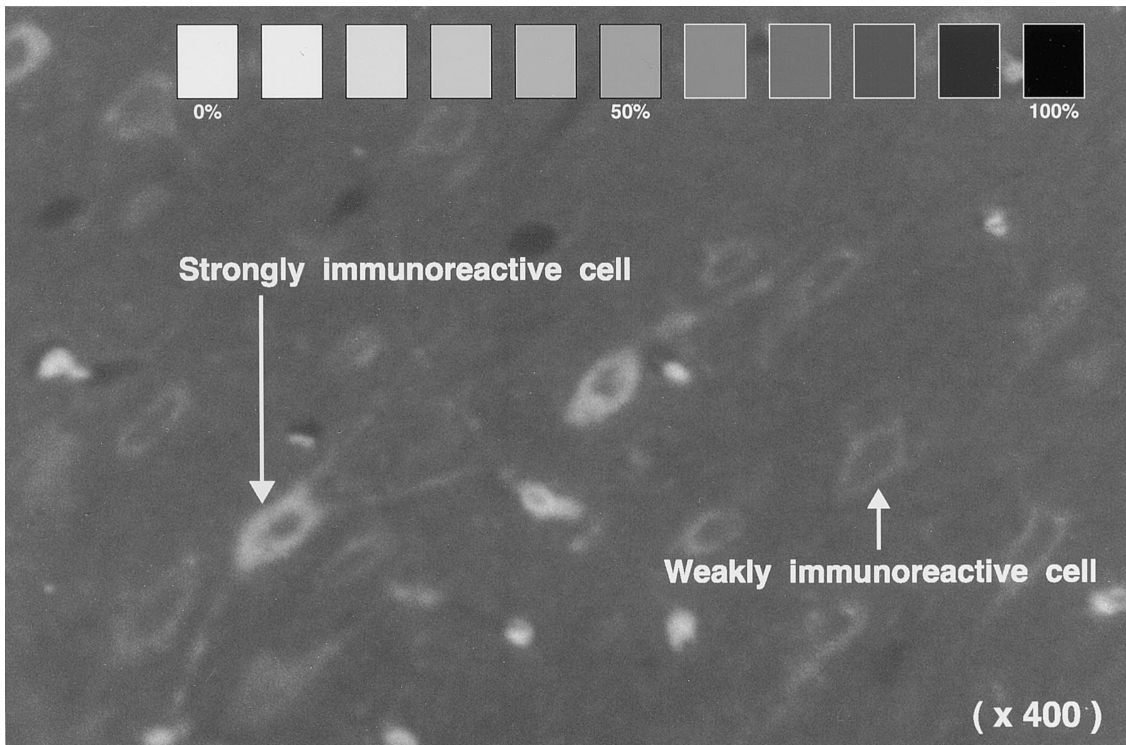

FIG. 3. Typical strongly and weakly immunoreactive nerve cells and the control image of their light absorption (%), with the most luminous part regarded as 0%.

3 g/kg ethanol), the number of weakly immunoreactive cells was elevated during the earlier period (group F of Fig. 1). Although individual discrepancies of the time of death were observed in each animal, the drug-induced disorders could be scored equally, regardless of the duration of each disorder, using the above criteria (Table 1). Serious respiratory disorders were not observed until near the time of death for all of the late fatalities observed in group F′ (score 1 disorders of Table 1). In contrast, the early deaths observed mainly in group C′ were accompanied by early serious respiratory disorders (score 2 disorders). The frequency of early deaths increased in group C′ compared to group F′, but this increase was not significant. The mean scores of respiratory disorders observed in Table 2 did not differ significantly between the two groups. All of the deaths in group C′ were accompanied by seizures with obvious tonic–clonic activity (score 2 or 1 seizures). In particular, serious seizures with obvious tonic–clonic activity (score 2 seizures) were observed in four of five animals in the early death group. Such tonic–clonic seizures were less obvious in group F′ (at most score 1 seizures), compared to group C′. In three animals in the late death group, no seizures were observed (score 0). In a preliminary experiment, groups following other protocols (the protocols used in groups B, D, and E) were also examined ($n = 4$), but the disorders de-

TABLE 1

CRITERIA FOR THE OBSERVED DISORDERS IN EXPERIMENT 2

(A) Survival times
- Early death: survival time less than 1 h (less than 20 min in all cases)
- Late death: survival time over 1 h (over 7 h in all cases)

(B) Respiratory disorders
- Score 0: none or slight
- Score 1: moderate or no obvious respiratory disorders until near the time of death
- Score 2: serious respiratory disorders

(C) Locomotive disturbances
- Score 0: none or slight
- Score 1: moderate or no obvious locomotive disturbances until near the time of death
- Score 2: serious locomotive disturbances

(D) Seizures
- Score 0: no seizures
- Score 1: moderate seizures
- Score 2: serious seizures with obvious tonic–clonic activity

TABLE 2

OBSERVED DISORDERS IN THE ADMINISTRATION OF THE HIGH COCAINE DOSE (75 mg/kg)

	Group C′ (n = 7)	Group F′ (n = 7)
Drug administration protocol		
Cocaine	75 mg/kg	75 mg/kg
Ethanol	0 g/kg	3 g/kg
Frequency of death (total)	85.7%	100.0%
Frequency of late death (>1 h)	14.3%	71.4%[A]
Respiratory disorders (mean score ± SD)	1.6 ± 0.7	1.3 ± 0.5
Locomotive disturbances (mean score ± SD)	1.6 ± 0.7	1.6 ± 0.5
Seizures (mean score ± SD)	1.4 ± 0.7	0.6 ± 0.5[a]

The observed disorders (respiratory disorders, locomotive disturbances, and seizures) were scored in seven rats (both dead and surviving rats) of each group according to the criteria in Table 1, and the mean average value of all seven rats was calculated and expressed as a mean score ± SD. The frequency of death was expressed as the percentage of dead rats over the total number of rats used. The characteristic distribution for the score of each disorder is explained in detail in the text. [A]Significant increase compared to Group C′. [a]Significant attenuation compared to Group C′.

scribed in Table 1 were not observed. Increased ambulation, as described in a previous report (14), was observed in the group B rats, but this generally decreased in the groups treated with 3 g/kg ethanol with or without 15 mg/kg cocaine (groups D and E of Fig. 1).

In Experiment 3, a lower dose (50 mg/kg) of cocaine was used than in Experiments 1 or 2 (75 mg/kg) to collect data relevant to delayed toxicity. This was done because almost all of the animals in Experiment 2 (75 mg/kg cocaine) died, and their delayed deaths occurred at different times. Even when the data were collected at 24 h after the cocaine administrations, the number of weakly immunoreactive cells was elevated (groups H and J of Fig. 2) compared to the groups without cocaine (groups G and I of Fig. 2). In the ethanol group without cocaine (group I) no significant increase or decrease was observed in the number of immunoreactive cells compared to the group without any drug administration (group G). However, in the cocaine–ethanol group (group J), the number of weakly immunoreactive cells was greater than in the cocaine-only group (group H) at 24 h after the cocaine administration, as was seen in Experiment 1 with the higher cocaine dose and the earlier perfusion time.

DISCUSSION

The present study demonstrated elevated levels of rat brain β-endorphin immunoreactivity caused by toxic doses of cocaine and combined cocaine–ethanol. The specific presence of elevated numbers of β-endorphin-immunoreactive cells following the administration of a toxic cocaine dose (75 mg/kg) suggests the possibility of using the immunoreactivity as an index of toxicity. In group F of Experiment 1, the cocaine–ethanol group subjected to 75 mg/kg cocaine plus 3 g/kg ethanol, the early elevated number of weakly immunoreactive cells seemed to be correlated with the late toxicities and the elevated frequency of late deaths, based on the results from Experiment 2. In addition, the results from Experiment 3, taken 24 h after cocaine or cocaine–ethanol administration, support the continued presence of weakly immunoreactive cells as a parameter for late fatal toxicity. In Experiment 3 (Fig. 2), a lower dose of cocaine (50 mg/kg) was used than in the other experiments (75 mg/kg) to determine the trends of the late data at the same time after the cocaine administration. Therefore, the results from the surviving rats in this experiment did not correspond precisely to the fatal toxicities. However, although the time of perfusion was shortened to collect data before the occurrence of death, the fragmentary data obtained from a preliminary study using 75 mg/kg cocaine support a relationship between late fatal toxicities and an increased number of weakly immunoreactive cells. When the perfusion was performed soon after the appearance of any symptoms of toxicity as explained in Experiment 2, an elevated number of weakly immunoreactive cells was observed, but the number of strongly immunoreactive cells did not increase. On the other hand, no fatalities were observed in group E, the cocaine–ethanol group subjected to a lower dose of cocaine (15 mg/kg) plus 3 g/kg ethanol, even though the total number of immunoreactive cells was not significantly different from group C, the cocaine-only group subjected to 75 mg/kg cocaine, in which cases of fatal toxicity were observed. In addition, the number of weakly immunoreactive cells was rather enhanced in group E compared to group C. Furthermore, even the late fatal toxicity was not observed in group E, despite the coadministration of 3 g/kg ethanol. A comparison of groups C, E, and F revealed the presence of a considerable number of early, strongly immunoreactive cells in the groups in which any fatalities were observed (groups C and F). The lower number of strongly immunoreactive cells in group E compared to groups C or F suggests the existence of a threshold value for the number of strongly immunoreactive cells in those cases of fatality. Furthermore, the increased number of strongly immunoreactive cells only in group C could be independently correlated with the occurrence of early fatalities. However, judging from the decreased number of strongly immunoreactive cells at 24 h after the cocaine administration in Experiment 3, the continued presence of these cells might not be necessary for the late fatal toxicities. The decrease in the proportion of early deaths in group F′ was not significant, but some early protective effect caused by ethanol might be suspected; this effect could be correlated with the elevated number of weakly immunoreactive cells observed in Experiment 1. Even though the number of strongly immunoreactive cells was also elevated at an early stage in group C (Experiment 1), in which the frequency of early deaths was elevated (Experi-

ment 2), the enhancement of weakly immunoreactive cells seemed to be individually correlated with the enhancement of late deaths. With respect to the underlying mechanisms of this enhanced occurrence of late deaths in rats treated with cocaine and ethanol, the delayed metabolism and toxic effects of cocaine caused by ethanol coadministration have been demonstrated (26–28). Therefore, any continuous protective effect due to ethanol can be excluded.

Regional differences in cell type between the rostral and caudal part of the arcuate nucleus have been previously reported (4,20), and more than one type of cell with different responses to hormones like androgens are believed to be present. The present study differentiated immunoreactive cells in the rat brain only visually, based on their light absorption values (%), and did not determine the specificity of any particular cell type. However, in human peripheral blood mononuclear cells, a source of β-endorphin in humans, the secretion mechanisms of β-endorphin have been differentiated by the different types of protein kinases activated (16). Furthermore, one type of immunoreactive blood cell, which was correlated with protein kinase-C activation, was activated rapidly, whereas delayed and moderately elevated immunoreactivity was observed in another type of cell that was correlated with protein kinase-A as well as protein kinase-C activation. Although the results from these experiments using human peripheral blood mononuclear cells cannot be simply applied to the present experiment using rat brain nerve cells, it is possible that the delayed activation of a specific group of brain cells is mediated by a particular mechanism that was correlated with the delayed toxicities of these drugs.

Of the disorders observed in the rats treated with a toxic dose of cocaine (75 mg/kg), only the seizure score was influenced by the coadministration of ethanol (Experiment 2). The absence of seizures with obvious tonic–clonic activity in those rats treated with both cocaine and ethanol (group F′ in Experiment 2) suggests that the number of β-endorphin immunoreactive cells, especially weakly immunoreactive cells, is not always correlated with the occurrence of cocaine-induced seizures. The elevated frequency of late deaths seen in the above group seems to reflect an enhancement of drug toxicity, which is independent of the seizure activity due to the coadministration of ethanol. Previous reports have demonstrated a relationship between seizures, especially serious convulsive seizures, and elevated levels of β-endorphin, and have also suggested that a type of cocaine-induced toxicity was correlated with the severity of the seizures (24,32). However, another type of cocaine-induced toxicity independent of seizure activity, especially obvious tonic–clonic activity, has also been demonstrated (19,32). The elevated frequency of late deaths seen in group F′ (Table 2) seems to reflect an enhancement of this latter type of toxicity induced by the coadministration of ethanol. In this type of drug toxicity, alterations in β-endorphin levels have not been previously examined. However, judging from the results of the present experiments, the continued presence of an increased number of weakly immunoreactive cells or a type of enhanced secretion of β-endorphin seems to be correlated with the type of cocaine (plus ethanol)-induced toxicity independent of seizure activity. Therefore, the present study demonstrated two types of coaine (plus ethanol)-induced toxicity, which can be differentiated by the different types of brain β-endorphin immunoreactivity; one type of toxicity correlated with seizure activity and early fatalities, in which the number of strongly immunoreactive cells was immediately increased, and a second type of toxicity correlated with late fatalities, which was independent of seizure activity and in which the number of weakly immunoreactive cells was continuously increased. The scores for the other disorders observed in Experiment 2 did not differ significantly between the two groups when the mean values of both dead and surviving rats were compared. However, judging from the different distribution of each score as described in the results, a more subtle relationship between the types of immunoreactivity and the types of toxicity, including the toxic effects in the surviving rats (ex. depressed behaviors provided by ethanol) may exist and needs further consideration.

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