

Effects of Eptazocine, a Novel Analgesic, on KCN-Induced Changes in the Cerebral Contents of Glycolytic Metabolites and High-Energy Phosphates in Mice

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Accepted July 10, 1990

Abstract—Effects of eptazocine on cerebral metabolic changes due to a sublethal dose of KCN were investigated in mice. KCN (2 mg/kg, i.v.) induced a temporary loss of consciousness being moderated by eptazocine (1–10 mg/kg) in a dose-dependent manner. The KCN injection decreased the contents of phosphocreatine (PCr), ATP and glucose and increased the contents of AMP and lactate, resulting in a 34% decrease in energy charge potential (ECP) and an increase in lactate/pyruvate (L/P) ratio. Such changes were improved by eptazocine (10 mg/kg) and EKC (3 mg/kg), but not by pentazocine (10 mg/kg) and morphine (3 mg/kg), and the improving effect of eptazocine was completely inhibited by MR-2266 (3 mg/kg), a relatively selective opioid κ -receptor antagonist. On the other hand, eptazocine (3, 10 mg/kg) was found to increase the glucose content in normal mice, but not to give significant changes in the contents of glycolytic metabolites and high-energy phosphates. These results suggest that eptazocine may improve anoxic changes in cerebral energy metabolism.

Eptazocine (*(1,4-dimethyl-10-hydroxy-2,3,4,5,6,7-hexahydro-1,6-methano-1H-4-benzazonine)*) has been regarded as such an opioid μ -antagonist- κ -agonist, like pentazocine (1–3). In our previous study (4), analgesic doses of eptazocine significantly prolonged the survival time of mice subjected to either hypobaric hypoxia or KCN-induced cytotoxic anoxia in a dose-dependent manner; and the effect was inhibited by MR-2266, a relatively selective opioid κ -receptor antagonist, more potently than by naloxone, suggesting that eptazocine may elicit its anti-anoxic action via its binding with opioid κ -receptors. Beneficial effects of opioid κ -agonists against ischemic neurological deficits in experimental animals were reported (5–7). Actions of these compounds, however, on cerebral energy metabolism, which is extremely sensitive to anoxic and ischemic treatments, have not been elucidated. It is of great interest to determine whether eptazocine

may improve anoxic changes in cerebral energy metabolism through opioid κ -receptors. In the present study, effect of eptazocine on cerebral metabolic changes due to an injection of a sublethal dose of KCN (2 mg/kg, i.v.) were investigated in comparison with those of typical opioid compounds.

Materials and Methods

Animals: Male mice of the ddY strain (Nihon SLC Co., Ltd., Shizuoka), weighing 26–32 g, were used. They were housed in an air-conditioned room at $23 \pm 1^\circ\text{C}$ and were given standard diet (MF, Oriental Yeast Co., Ltd., Tokyo) and tap water ad libitum before being used.

Drugs: Eptazocine hydrobromide, ethyl-ketocyclazocine methanesulfonate (EKC) and (–)-2-(3-furylmethyl)-5,9-diethyl-2'-hydroxy-6,7-benzomorphan (MR-2266) were synthesized in our laboratories. Pentazocine hydrochloride and morphine hydrochloride

were purchased from Sankyo Co., Ltd. (Tokyo); glucose-6-phosphate (G6P), fructose-1,6-diphosphate (FDP), hexokinase, G6P dehydrogenase, pyruvate kinase, myokinase, amyloglucosidase, adenine dinucleotides and adenosine phosphates were from Sigma Chemical Co. (St. Louis, MO, U.S.A.); and lactate dehydrogenase and creatine kinase were from Boehringer Mannheim Yamanouchi Co., Ltd. (Tokyo). Other chemicals were obtained from Wako Pure Chemical Industries, Ltd. (Osaka). MR-2266 was dissolved in 100 mM methanesulfonate and diluted with distilled water. Other test drugs were dissolved in physiological saline.

Loss of consciousness due to a KCN injection: Mice pretreated with test drugs were injected with KCN (2 mg/10 ml/kg) at the rate of 0.6 ml/10 sec via the tail veins, and durations of loss of the righting reflex and spontaneous movement were measured, respectively.

Determination of glycolytic metabolites and high-energy phosphates: Mice pretreated with test drugs and/or KCN (2 mg/kg, i.v.) were sacrificed by microwave irradiation (TMW-6402; 5 kW, 1.4 sec, Toshiba). The whole brains were taken out after the heads were cooled in ice-cold water. The brains were weighed, and homogenized in 3.5 ml of 2 N perchloric acid. After each portion of the homogenate was taken for glycogen analysis (8), the residuals were allowed to stand in ice-cold water for 30 min and then centrifuged at 20,000×g for 15 min. The supernatants were neutralized with 6 N K₂CO₃, centrifuged at 1,000×g for 10 min, and used for the assays. PCr, ATP, glucose, pyruvate and lactate were analyzed according to the enzymatic fluorometric techniques as de-

scribed by Lowry et al. (9); and G6P, FDP, ADP and AMP were determined by the UV-methods of Bergmeyer (10–12). The energy state of each tissue was expressed as the energy charge potential (ECP) of the adenine nucleotide pool according to Atkinson (13): $ECP = [(ATP) + 0.5(ADP)] / [(ATP) + (ADP) + (AMP)]$.

Statistical analysis: Values were represented as the mean±S.E., and one-way analysis of variance followed by Student's *t*-test or the Aspin-Welch *t*-test were carried out.

Results

Effect of eptazocine on loss of consciousness due to a KCN injection: An injection of KCN (2 mg/kg, i.v.) to saline-treated mice immediately induced clonic convulsion, followed by loss of the righting reflex with a respiratory failure; and about 342 sec later, the mice had spontaneous movement again. Effects of eptazocine (1–10 mg/kg, i.p.) on KCN-induced loss of the righting reflex and spontaneous movement were examined, and the results are shown in Table 1. Eptazocine (10 mg/kg) shortened the duration of loss of consciousness.

Effects of eptazocine and opioid compounds on changes in the contents of cerebral metabolites due to the KCN injection: The preliminary experiments showed that the cerebral ATP content in mice decreased to 82, 55 and 74% of the normal value 45, 90 and 180 sec after the KCN injection, respectively. Therefore, the contents of cerebral metabolites, ECP and L/P ratio were determined 90 sec after the KCN injection (Tables 2 and 3). The cerebral contents of PCr and ATP were decreased, and the AMP content

Table 1. Effect of eptazocine on KCN-induced loss of consciousness in mice

Treatment	Dose (mg/kg, i.p.)	RR time (sec)	SM time (sec)
Control	—	260±28	342±30
Eptazocine	1	233±25	290±25
	3	188±22	267±25
	10	181±11*	248±24*

Each value represents the mean±S.E. (n=8). *P<0.05, compared with each control value. KCN (2 mg/kg, i.v.) was injected 30 min after administration of saline (control) or eptazocine. RR time: duration of loss of righting reflex. SM time: duration of loss of spontaneous movement.

Table 2. Effects of eptazocine, EKC, pentazocine and morphine on KCN-induced changes in the cerebral contents of PCr, ATP, ADP and AMP in mice

Treatment	Dose (mg/kg, i.p.)	Contents in cerebral tissue (μ mole/g)				ECP
		PCr	ATP	ADP	AMP	
Normal	—	5.30 \pm 0.18**	2.53 \pm 0.04**	0.53 \pm 0.03	0.06 \pm 0.01**	0.897 \pm 0.005**
Control	—	2.70 \pm 0.28	1.26 \pm 0.14	0.59 \pm 0.05	0.75 \pm 0.06	0.590 \pm 0.034
Eptazocine	10	4.43 \pm 0.26**	2.07 \pm 0.17**	0.56 \pm 0.04	0.36 \pm 0.13*	0.790 \pm 0.049**
EKC	3	3.60 \pm 0.27*	2.01 \pm 0.18**	0.55 \pm 0.01	0.31 \pm 0.10**	0.788 \pm 0.043**
Pentazocine	10	2.43 \pm 0.40	1.44 \pm 0.21	0.57 \pm 0.02	0.68 \pm 0.16	0.635 \pm 0.069
Morphine	3	2.34 \pm 0.26	1.30 \pm 0.18	0.62 \pm 0.04	0.83 \pm 0.15	0.584 \pm 0.060

Each value represents the mean \pm S.E. (n=7). *P<0.05, **P<0.01, compared with each control value. KCN (2 mg/kg, i.v.) was injected 30 min after administration of saline (control) or test drugs. Mice received microwave irradiation (5 kW, 1.4 sec) 90 sec after the KCN injection. PCr: phosphocreatine ECP: energy charge potential.

Table 3. Effects of eptazocine, EKC, pentazocine and morphine on KCN-induced changes in the cerebral contents of glucose, lactate and pyruvate in mice

Treatment	Dose (mg/kg, i.p.)	Contents in cerebral tissue (μ mole/g)			L/P
		Glucose	Lactate	Pyruvate	
Normal	—	1.84 \pm 0.09**	1.24 \pm 0.13**	0.091 \pm 0.008	13.8 \pm 0.8**
Control	—	0.45 \pm 0.03	7.92 \pm 0.20	0.109 \pm 0.005	73.5 \pm 3.3
Eptazocine	10	1.09 \pm 0.12**	6.22 \pm 0.40**	0.148 \pm 0.015	45.5 \pm 5.9**
EKC	3	0.77 \pm 0.04**	7.43 \pm 0.32	0.145 \pm 0.015	54.6 \pm 5.7*
Pentazocine	10	0.39 \pm 0.03	8.47 \pm 0.37	0.123 \pm 0.005	68.9 \pm 2.5
Morphine	3	0.44 \pm 0.03	8.02 \pm 0.26	0.115 \pm 0.006	71.8 \pm 6.7

Each value represents the mean \pm S.E. (n=7). *P<0.05, **P<0.01, compared with each control value. KCN (2 mg/kg, i.v.) was injected 30 min after administration of saline (control) or test drugs. Mice received microwave irradiation (5 kW, 1.4 sec) 90 sec after the KCN injection. L/P: lactate/pyruvate ratio.

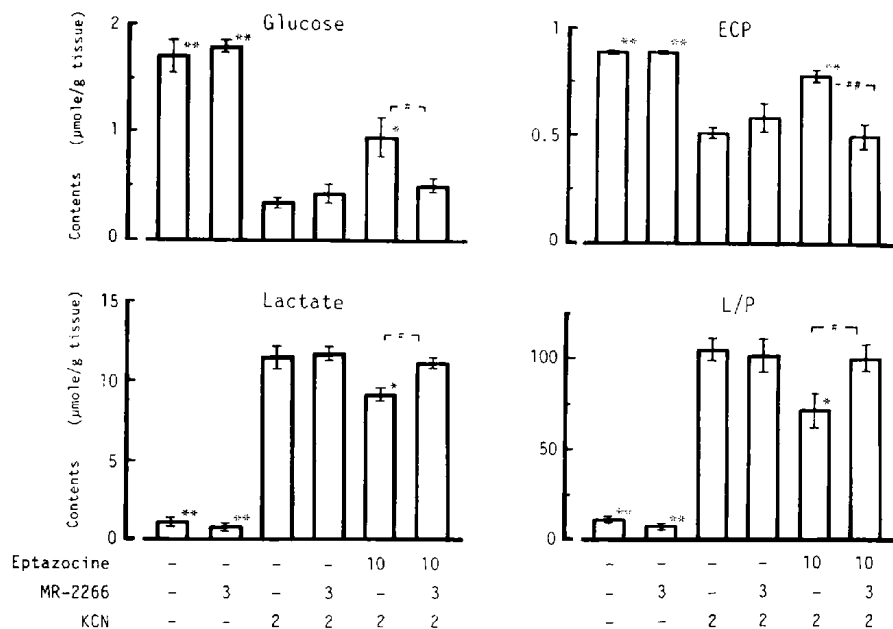


Fig. 1. Antagonistic effect of MR-2266 on the improving effect of eptazocine against KCN-induced cerebral metabolic changes in mice. Each column with vertical bars represents the mean \pm S.E. ($n=6$). * $P<0.05$, ** $P<0.01$, compared with each KCN alone value. # $P<0.05$, ## $P<0.01$, compared with each (KCN plus eptazocine) value. Eptazocine and MR-2266 were administered i.p. at 20 and 30 min before a KCN (2 mg/kg, i.v.) injection, respectively. Mice received microwave irradiation (5 kW, 1.4 sec) 90 sec after the KCN injection.

was increased, resulting in a decrease in ECP. The cerebral anoxic treatment also showed a decrease in the glucose content and increases in the lactate content and L/P ratio. These KCN-induced metabolic changes were prevented by a 30 min-pretreatment with eptazocine (10 mg/kg) or EKC (3 mg/kg), whereas morphine (3 mg/kg) and pentazocine (10 mg/kg) did not show such effects. MR-2266 was used to determine whether such effects of eptazocine were mediated via opioid κ -receptors or not. MR-2266 of 3 mg/kg, which showed no significant effect on the cerebral energy metabolism, completely inhibited the effects of eptazocine (Fig. 1).

Effects of eptazocine and opioid compounds on the contents of cerebral glycolic metabolites in normal mice: We attempted to determine whether the above-mentioned effects of eptazocine are shown in normal mice. Tables 4 and 5 show that a 30-min pretreatment with eptazocine induced an increase in the glucose content but no changes

in the contents of glycogen, G6P, FDP, PCr, ATP, lactate and pyruvate. An increase in the glucose content was also shown in the case of EKC (3 mg/kg) and morphine (3 mg/kg).

Discussion

Cyanide inhibits the terminal oxidase enzyme cytochrome oxidase in the cerebral tissue, resulting in disorder of the mitochondrial energy-producing systems (14–16), and such a cyanide-induced acute cerebral anoxia in animals has been widely used for the evaluation of cerebral protective drugs (17–19). In the previous report (4), we showed that eptazocine as well as EKC, a typical opioid κ -agonist, prolonged the survival time of mice subjected to a lethal dose of KCN (3 mg/kg, i.v.). In this study, a sublethal dose of KCN (2 mg/kg, i.v.) was tested, and KCN-induced temporary loss of consciousness was shown to be overcome by the pretreatment with eptazocine. In order to elucidate whether such an effect of eptazocine can be at-

Table 4. Effects of eptazocine, EKC, pentazocine and morphine on the cerebral contents of glycogen, glucose, G6P and FDP in mice

Treatment	Dose (mg/kg, i.p.)	Contents in cerebral tissue (μ mole/g)			
		Glycogen	Glucose	G6P	FDP
Normal	—	2.42 \pm 0.15	1.73 \pm 0.10	0.135 \pm 0.004	0.134 \pm 0.014
Eptazocine	3	2.20 \pm 0.12	2.14 \pm 0.10*	0.137 \pm 0.004	0.154 \pm 0.014
	10	2.09 \pm 0.12	2.49 \pm 0.07**	0.138 \pm 0.004	0.131 \pm 0.014
EKC	3	2.50 \pm 0.14	2.76 \pm 0.14**	0.140 \pm 0.006	0.131 \pm 0.012
Pentazocine	10	2.28 \pm 0.09	1.79 \pm 0.07	0.136 \pm 0.004	0.160 \pm 0.012
Morphine	3	2.28 \pm 0.13	2.05 \pm 0.10*	0.136 \pm 0.003	0.114 \pm 0.010

Each value represents the mean \pm S.E. (n=7). *P<0.05, **P<0.01, compared with each normal value. Mice received microwave irradiation (5 kW, 1.4 sec) 30 min after administration of test drugs. G6P: glucose-6-phosphate. FDP: fructose-1,6-diphosphate.

Table 5. Effects of eptazocine, EKC, pentazocine and morphine on the cerebral contents of PCr, ATP, lactate and pyruvate in mice

Treatment	Dose (mg/kg, i.p.)	Contents in cerebral tissue (μ mole/g)				L/P
		PCr	ATP	Lactate	Pyruvate	
Normal	—	5.04 \pm 0.16	2.43 \pm 0.06	1.29 \pm 0.04	0.144 \pm 0.013	9.80 \pm 1.54
Eptazocine	3	4.48 \pm 0.50	2.37 \pm 0.11	1.09 \pm 0.01	0.134 \pm 0.007	8.09 \pm 0.64
	10	5.73 \pm 0.34	2.64 \pm 0.08	1.17 \pm 0.06	0.149 \pm 0.010	7.97 \pm 0.47
EKC	3	4.49 \pm 0.46	2.43 \pm 0.09	1.18 \pm 0.06	0.137 \pm 0.007	8.80 \pm 0.69
Pentazocine	10	5.23 \pm 0.59	2.60 \pm 0.06	1.42 \pm 0.08	0.152 \pm 0.012	9.84 \pm 1.24
Morphine	3	5.39 \pm 0.30	2.59 \pm 0.06	1.34 \pm 0.12	0.154 \pm 0.011	8.92 \pm 1.08

Each value represents the mean \pm S.E. (n=7). Mice received microwave irradiation (5 kW, 1.4 sec) 30 min after administration of test drugs. L/P: lactate/pyruvate ratio.

tributed to an improvement by eptazocine of cerebral energy metabolism, KCN-induced changes in cerebral energy metabolism were first investigated using biochemical techniques. At 90 sec after the KCN injection, the cerebral tissue was in a severe anoxic state, since ECP, which is a parameter of the cerebral energy state (13, 20), decreased from 0.897 to 0.590. The L/P ratio also increased from 13.8 to 73.5, indicating an increase in the anaerobic glycolytic rate. These KCN-induced changes in cerebral energy metabolism were prevented by eptazocine (10 mg/kg), suggesting an improving action of eptazocine on the cerebral glycolytic pathway or a mitochondrial respiratory function. The action of eptazocine seems likely to be mediated via opioid κ -receptors, because it was completely inhibited by MR-2266 (3 mg/kg), which has been reported to be a relatively selective κ -receptor antagonist rather than a μ -antagonist (21). Moreover, EKC showed a similar effect, whereas morphine (3 mg/kg) and pentazocine (10 mg/kg) had no significant effect, supporting our previous report concerning prolonged effects on the survival time in mice subjected to cerebral anoxia (4).

High doses of eptazocine showed non-specific inhibitory actions on the central nervous system in mice (22). Barbiturates (23–25), diazepam (26) and nifedipine (27) induced anti-anoxic effects through reducing the cerebral metabolic rate. The administration of eptazocine at 3 or 10 mg/kg to normal mice was found to increase the cerebral glucose content, but this caused no significant changes in the contents of glycolytic intermediates. At present, the mechanism by which eptazocine increased the glucose content is not clear, but eptazocine seems to show no inhibitory effect on the cerebral glycolytic metabolism, different from what is reported in the case of the above-mentioned drugs. In addition, EKC and morphine also increased the glucose content. It remains to be determined whether such an glucose increase can contribute to improvement of the anoxic changes.

In conclusion, the protective effects of eptazocine on cerebral anoxia may be attributed to the improvement of anoxic

changes in cerebral energy metabolism.

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