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The Role of H1- and H2-Receptors in the Effect of Compound 48/80 in the Asphyxiation and Body Temperature of Mice

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Contribution of histamine H1- and H2-receptors to the effect of compound 48/80, a potent histamine releaser, upon asphyxiation and body temperature in mice was investigated in the present experiments. Compound 48/80 showed an apparent protective potency against hypoxia and significantly prolonged the latencies for convulsions and death in a dose-dependent manner. Compound 48/80 also decreased the body temperature, which was in relation with the antihypoxic effect. Both the H1-receptor antagonist, dimethindene, and the H2-receptor antagonist, ranitidine, attenuated the hypothermic effect of compound 48/80, indicating the involvement of central histamine through both the H1- and H2-receptors. Ranitidine had no effect on the protective effect of compound 48/80 against hypoxia-induced lethality, whereas dimethindene completely antagonized it. These results suggest that the protective effect of compound 48/80 against hypoxia is mediated through histamine H1-receptors and is not related to its ability to induce hypothermia.

Key Words: Compound 48/80, histamine, hypoxia, body temperature, histamine receptors

Brain histamine is localized in both neurons and mast cells and there is direct biochemical evidence that mast cells contribute to brain histamine levels (Schwartz, 1975; Goldschmidt *et al.* 1985; Lewis *et al.* 1986). Compound 48/80 is known to be a potent histamine liberator and has been used widely in experiments concerning the physiological importance of histamine (Paton, 1951; Brashear *et al.* 1970). Some of the effects of compound 48/80 on the brain have been recently studied. It's effects on behavior include head and body shakes, paw tremor, grooming, unusual posture, mild diarrhoea, piloerection, sedation and catatonia. Compound 48/80 decreased the histamine concentrations in almost all brain regions and the noradrenaline concentrations in the cerebellum, hypothalamus and medulla oblongata-pons, although the dopamine content was decreased only in the medulla oblongata-pons. Compound 48/80 did not affect the concentration of serotonin and made a considerable rise in serum corticosterone level (Lewis *et al.* 1986; Bugajski *et al.* 1994).

Evidence also suggests that biogenic amins such as histamine, serotonin and dopamine, which are stored and released by mast cells, serve physiological roles as neuromodulators of brain functions (Dropp, 1972; Ibrahim, 1974; Lewis *et al.* 1986). Although histamine is now widely accepted as a transmitter or modulator in the central nervous system, it is only recently that investigations on its function have been undertaken (Schwartz *et al.* 1980; Lewis *et al.* 1986; Scherkl *et al.* 1991).

Although the function of histamine and histamine receptors in hypoxia has been recently

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observed, there is conflicting evidence (Dux *et al.* 1987; Waskiewicz *et al.* 1988; Audibert *et al.* 1991; Shibata and Watanabe, 1993). Hypothermia is known to protect animals from hypoxia and cerebral ischemia (Smith, 1977; Steen and Mitchenfelder, 1979; King, 1987a; King, 1987b). Minard and Grant have reported that protection from hypoxia by drugs can be completely accounted for by drug-induced hypothermia (Minard and Grant, 1982), whereas other investigators suggest that some drugs have protective effects against hypoxia which are independent of drug-induced hypothermia (King, 1987a; King, 1987b; Ulugöl *et al.* 1995).

In the present study, we observed the role of histamine H1- and H2-receptors in the effect of 48/80 on hypoxia-induced convulsions and death in relation to its hypothermic effect on mice.

MATERIALS AND METHODS

Animals

Male Bulb/c albino mice (Eczacybasi, Turkey) weighing 25~30 g were used. The animals were housed at constant room temperature (22±1℃), with food and water *ad libitum*, and a 12 hr light/dark cycle (lights on at 6:00 A. M. and off at 6:00 P.M.). Because there is an age-dependent increase of histamine content in mast cells (Okudaria *et al.* 1980), all mice used were 4 months old.

Measurement of rectal temperature

The temperature was measured to the nearest 0.1℃ by an Ellab thermometer. This was done by inserting the probe (2 mm diameter) for 2.5 cm into the rectum of the mice. The probe was left in place until steady readings were obtained (20-25 s).

Experimental procedure

The animals were weighed, injected with a compound 48/80 solution intraperitoneally and rectal temperatures were taken in groups of 6. Twenty min after the first measurement and 2 min prior to hypoxia, rectal temperatures were taken for the second time. Antagonists (dimethindene and ranitidine) were injected 10 min before the compound 48/80 injection. The animals were asphyxiated by putting them individually into a tightly closed glass container of 300 ml capacity. The animals had convulsions and died due to hypoxia, or lack of oxygen. The latencies for death were noted as described previously (Bharvaga, 1986; Kunchandy and Kulkarni, 1988; Ulugöl *et al.* 1995). It was very difficult to determine the exact time of death, although the animals died approximately 2 min after they had convulsions. Every effort was made to determine the exact time of death. Parallel control studies were run; the control animals were also weighed, injected with 0.1 ml/10 g NaCl solution intraperitoneally, and rectal temperatures were taken (n=6). Rectal temperatures were taken for the second time twenty min after the first measurement, and then the animals were asphyxiated as noted above.

A limitation of the experimental method used here is that body temperatures were measured prior to hypoxic exposure for practical reasons. However, all the treatments, including hypothermia, were affected in a similar manner.

The experiments had been approved by the "Center of the Laboratory Animals - Animal Care Ethics Committee" of our faculty.

Drugs

Dimethindene (Fenistil®, Ciba-Geigy, Istanbul, Turkey) and ranitidine (Ulcuran®, Abfar-Zyma, Istanbul, Turkey) were diluted from commercial preparations. Compound 48/80 was a gift from Prof. Dr. A. Akçasu, Department of Pharmacology, Cerrahpapa Medical Faculty of Istanbul University. All chemicals were dissolved in isotonic NaCl and administered intraperitoneally in a volume of 0.1 ml/10 g body weight. The control group received 0.1 ml/10 g NaCl solution intraperitoneally.

Statistical analysis

Results were evaluated by ANOVA followed by the Duncan multiple-range test. To observe the relationship of the latencies for death to the decrease in body temperature, correlation analysis were performed. Values are expressed

as means±S.E.M.

RESULTS

Effect of hypoxia

All mice showed an increased respiratory rate, tremors and convulsions followed by death with the induction of suffocation. An increase in urination and defecation were also observed. Control animals developed convulsions within 25.22±0.80 and died within 26.80±0.86 min (Fig. 1, 2).

Effect of compound 48/80 on hypoxia-induced convulsions and death

Pretreatment with compound 48/80 (0.1～2 mg/kg), 20 min prior to exposure to hypoxia, dose-dependently increased the latencies for convulsions (30.43±0.98～49.96±2.76, $p<0.05$) and for death (31.60±1.54～51.80±3.52, $p<0.05$) (Fig. 1).

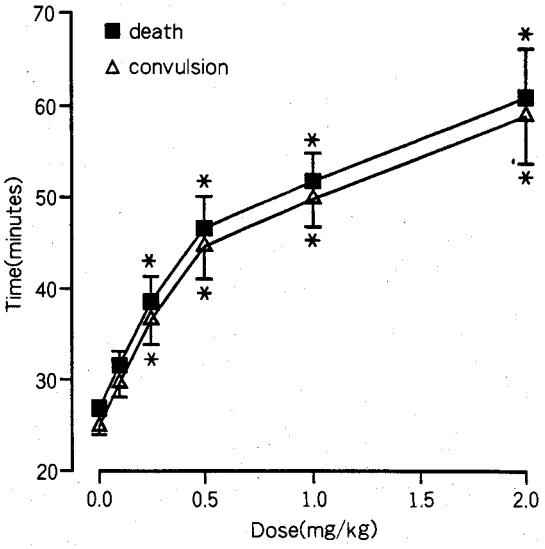

Fig. 1. *Effects of various doses of compound 48/80 on hypoxia-induced convulsions and death in mice.*
**: $p < 0.05$ vs saline using ANOVA followed by the Duncan multiple-range test. Each point is the mean value with S.E.M.(the bars).*

Relationship between latencies in death and body temperature

All doses of compound 48/80 decreased body temperature apparently and dose-dependently (36.95±0.31～35.33±0.39, $p<0.05$) (Fig. 3). There was a significant correlation between the decrease in body temperature and latencies in convulsions ($r=-0.68$, $p<0.05$) and death ($r=-0.70$, $p<0.05$).

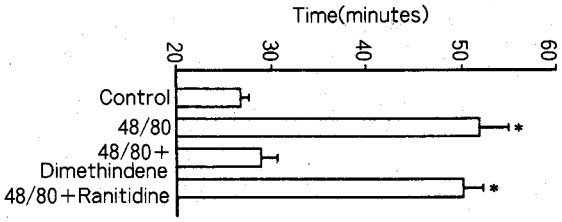

Fig. 2. *Effects of dimethindene(0.1 mg/kg, i.p.) and ranitidine(100 mg/kg, i.p.) on the antihypoxic effect of compound 48/80(1 mg/kg, i.p.) in mice.*
**: $p < 0.05$ vs saline; **: $p < 0.05$ vs compound 48/80, using ANOVA followed by the Duncan multiple-range test. Each point is the mean value with S.E.M.(the bars).*

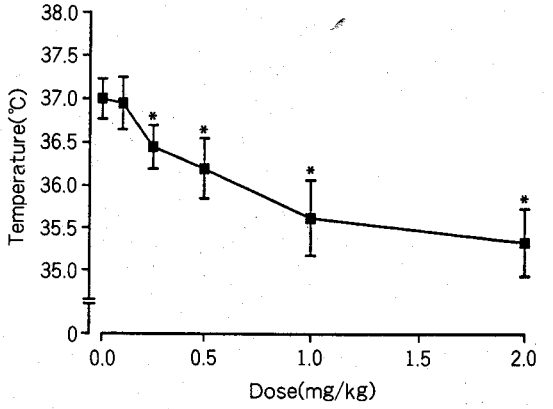

Fig. 3. *Effects of various doses of compound 48/80 on body temperature in mice.*
**: $p < 0.05$ vs saline, using ANOVA followed by the Duncan multiple-range test. Each point is the mean value with S.E.M.(the bars).*

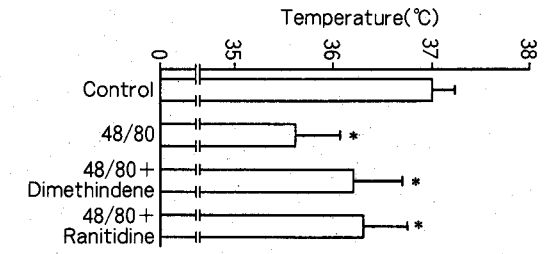

Fig. 4. *Effects of dimethindene(0.1 mg/kg, i.p.) and ranitidine(100 mg/kg, i.p.) on the hypothermic effect of compound 48/80(1 mg/kg, i.p.) in mice.*
**: $p < 0.05$ vs saline; **: $p < 0.05$ vs compound 48/80, using ANOVA followed by the Duncan multiple-range test. Each point is the mean value with S.E.M.(the bars).*

Effects of dimethindene and ranitidine

Both the H1-receptor antagonist, dimethindene (0.1 mg/kg), and H2-receptor antagonist, ranitidine (100 mg/kg), attenuated the hypothermic effect of compound 48/80 (1 mg/kg) ($p < 0.05$, Fig. 4). On the other hand, ranitidine had no effect on the protective effect of compound 48/80 against suffocation ($p > 0.05$), whereas dimethindene completely antagonized it ($p < 0.05$, Fig. 2). 1 mg/kg dose of compound 48/80 was chosen to use in combination with the antagonists, because of its reliable results in our earlier studies.

DISCUSSION

Several studies have demonstrated direct evidence that mast cells exist in the mammalian brain (Dropp, 1972; Ibrahim, 1974; Lewis *et al.* 1986); moreover, the roles of mast cells in the central nervous system in defensive inflammatory responses and tissue repair, detoxification of toxins, myelination and the metabolism of sulphate and lipids have been proposed (Dropp, 1972). Evidence also suggests that mast cells stores of histamine contribute significantly to the overall histamine content of the brain (Schwartz, 1975; Goldschmidt *et al.* 1985; Lewis *et al.* 1986). The transmitter role of histamine in the mammalian brain has been suspected for a long time, and there is considerable biochemical and electrophysiological evidence to support this idea (Schwartz *et al.* 1980; Lewis *et al.* 1986; Scherkl *et al.* 1991). The neurotransmitter and/or neuromodulator role of histamine in the regulation of neuroendocrine and neuroimmune functions, circadian rhythms, sleep-wakefulness cycle, body temperature, centrally-mediated neurovegetative functions, cerebrovascular control, and behavior and learning has been suggested (Lewis *et al.* 1986; Cacabelos, 1990; Scherkl *et al.* 1991).

Recent studies indicate that histamine plays a role during hypoxia (Dux *et al.* 1987; Waskiewicz *et al.* 1988; Audibert *et al.* 1991; Shibata and Watanabe, 1993). Hypoxia and ischemia modify histamine metabolism and transport in brain synaptosomes (Waskiewicz *et al.* 1988). H2-receptor blockers could interfere with the adaptation of cerebral blood flow during hypoxia (Audibert *et al.* 1991). Dux *et al* showed the protective role of both H1- and H2-receptor antagonists against general hypoxemia (Dux *et al.* 1987), and Shibata and Watanabe showed the neuroprotective effect of H1-receptor antagonists on ischemia-induced decrease in 2-deoxyglucose uptake in rat hippocampal slices (Shibata and Watanabe, 1993). Moreover, histamine is known to cause potent cerebral vazodilatation, which is one of the mechanisms responsible from hypoxia (Clozel *et al.* 1985; Wahl, 1985). In contradisfunction, our results indicate that compound 48/80 protects animals from hypoxia in a dose dependent manner. The H1-receptor antagonist, dimethindene (0.1 mg/kg), which is known to have few central side effects, completely antagonized the protective effect of compound 48/80, whereas the H2 receptor antagonist, ranitidine (100 mg/kg) had no effect, indicating the role of H1 receptors in the antihypoxic effect of compound 48/80. A high dose of ranitidine was used, since it is a poorly brain penetrating compund. Histamine H1- and H2-receptor antagonists were tested in doses that were used several times by other investigators and us (Gogas *et al.* 1989; Scherkl *et al.* 1991; Karadag *et al.* unpublished data; Ulugöl *et al.*

unpublished data).

Anoxia provokes convulsive seizures and increases the rate of oxygen utilization (Sharma *et al.* 1979). It has been postulated that prevention of hypoxia-induced seizures and/or reduction of cerebral metabolic rate are very important mechanisms in the antihypoxic effect (Hossmann, 1982). Histamine is known to antagonize seizures in experimental convulsions (Scherkl *et al.* 1991; Sen *et al.* 1991; Yokoyama *et al.* 1993; Karadag *et al.* unpublished data), and H2-receptor blockers interfere with the adaptation of cerebral blood flow during hypoxia (Audibert *et al.* 1991). Our results show that the anticonvulsive effect of compound 48/80 is more potent than the vazodilatator effect and that H1-receptors play a role in this method, since compound 48/80 increased the latencies for convulsions and death and only dimethindene antagonized this effect.

Hypothermia is known to have protective effects on hypoxia-induced death (Smith, 1977; Steen and Mitchenfelder, 1979; King, 1987a; King, 1987b). A decrease in the cerebral metabolic rate (Hagerdal *et al.* 1975) and an increase in the affinity of hemoglobin for oxygen (Carlsson *et al.* 1976) are the two mechanisms playing roles in the protective effects of hypothermia. Minard and Grant have indicated that drug-induced hypothermia is fully responsible for the protection from the lethal effects of hypoxia (Minard and Grant, 1982). On the other hand, some investigators have reported that protection from hypoxia cannot be accounted for by drug-induced hypothermia (King, 1987a; King, 1987b; Ulugöl *et al.* 1995). Several drugs, belonging to different pharmacological classes, physostigmine, oxotremorine, phenobarbital, diazepam, phenytoin, vinpocetine, moclobemide, etc., were found to have protective effects against hypoxia which are independent of drug-induced hypothermia (King, 1987a; King, 1987b; Ulugöl *et al.* 1995).

The effect of histamine on body temperature has been studied extensively (Dey and Mukhopadhaya, 1986; Hutchison and Spriesterbach, 1986; Mukhopadhayay and Dey, 1986; Fujimoto *et al.* 1990; Kandasamy and Hunt, 1990). Histamine may raise or lower body temperature depending on the dose (Hutchison and Spriesterbach, 1986), the environmental temperature (Dey and Mukhopadhaya, 1986; Fujimoto *et al.* 1990), and the route of administration (Mukhopadhayay and Dey, 1986). Hypothermia elicited from infusion of histamine into the lateral ventricle was prevented with pretreatment of H1-receptor antagonist, mepyramine, but in the case of the IVth ventricle, it was prevented with a H2-receptor antagonist, cimetidine (Dey and Mukhopadhaya, 1986). Our findings are consistent with these reports, since both of the antagonists attenuated the hypothermic effect of compound 48/80. Our statistical correlation analysis suggests that the antihypoxic effect of compound 48/80 is related to its ability to induce hypothermia. However, although there was a significant correlation between the decrease in body temperature and latencies in convulsions and death, this correlation does not seem to be a true direct relationship, since both histamine H1- and H2-receptor antagonists can diminish the hypothermic effect of compound 48/80 to similar extents, but only the histamine H1-receptor antagonist dimethindene antagonizes the antihypoxic effect.

These results suggest that although the hypothermic effect of compound 48/80 is mediated by both histamine H1- and H2-receptors, its protective effect against hypoxia-induced convulsions and death in mice is mediated mainly by H1-receptors and the hypothermic effect per se cannot account for the antihypoxic effect compound 48/80.

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