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The effects of choline on body temperature in conscious rats

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Abstract

Choline (75–300 μg) produced dose-dependent hypothermia when injected intracerebroventricularly (i.c.v.). Pre-treatment with the muscarinic receptor antagonist, atropine (10 μg, i.c.v.), blocked the hypothermic effect of choline (150 μg), but the response was only partially attenuated by pre-treatment with the nicotinic receptor antagonist, mecamylamine (20 μg, i.c.v.). Pirenzepine (25 μg), a muscarinic M_1 receptor antagonist, or hexahydro-siladifenidol (HHSD) (100 μg), a muscarinic M_3 receptor antagonist, also blocked choline-induced hypothermia when injected centrally. Unlike the other muscarinic receptor antagonists, M_2-selective 11-[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (AF-DX116) (10 μg), did not affect choline-induced hypothermia. We also found that choline-induced hypothermia was very sensitive to the ambient temperature. Similar to its effect at room temperature, choline produced dose-dependent hypothermia at 4°C, but this effect was abolished at 32°C. These data suggest that choline produces hypothermia and this effect is mediated by muscarinic receptors.

Keywords: Choline; Thermoregulation; Hypothermia; (Rat)

1. Introduction

Choline, the precursor of acetylcholine, is a major factor regulating acetylcholine synthesis and release. Treatments that increase extracellular choline are effective for stimulating presynaptic acetylcholine synthesis and release (Blusztajn and Wurtman, 1983; Maire and Wurtman, 1985; Ulus et al., 1989; Koshimura et al., 1990; Johnson et al., 1992; Farber et al., 1993; Marshall and Wurtman, 1993; Buyukuysal et al., 1995). Choline-induced acetylcholine synthesis and release was shown to be functional, producing biological responses in postsynaptic neurons and endocrine cells (Cohen and Wurtman, 1976; Ulus and Wurtman, 1976; Savci et al., 1996a,b). In addition to increasing acetylcholine synthesis and release, choline itself is an acetylcholine receptor agonist and produces in vitro biological effects at high concentrations (Ulus et al., 1988). The pharmacological effects of this novel precursor have not been studied extensively, however. Our laboratory has recently begun to characterize some of choline's effects (Arslan et al., 1991; Ulus et al., 1995; Savci and Ulus, 1996; Savci et al., 1996a,b), but many such as the thermoregulatory effects, remain to be elucidated.

Acetylcholine and other acetylcholine receptor agonists such as carbachol, pilocarpine and oxotremorine produce hypothermia when injected intracerebroventricularly in various species (Lomax and Jenden, 1966; Chawla et al., 1975; Lin et al., 1980). Similarly, microinjection of acetylcholine into hypothalamic thermoregulatory centers increases neuronal firing frequency and causes comparable hypothermia (Lomax and Jenden, 1966; Knox et al., 1973). These pharmacological data, together with results of immunohistochemical studies demonstrating the presence of intrinsic cholinergic neurons in the hypothalamus, suggest that cholinergic neurons are involved in thermoregulation (Tago et al., 1987).

The beneficial effects of choline-containing drugs, such as citicoline, have been postulated and/or reported to have effects in experimental models of various conditions including ischemic and traumatic injuries of the nervous system, aging and Alzheimer's disease. The neuroprotective effect of citicoline in ischemia seems to make it especially promising as an effective drug for clinical applications (Weiss, 1995). Since hypothermia might be neuroprotective, characterization of choline's thermoregulatory effect is crucial for evaluating the effectiveness of choline-containing drugs in brain ischemia.

In this study, we tested whether choline influences body temperature since cholinergic agents produce hypothermia

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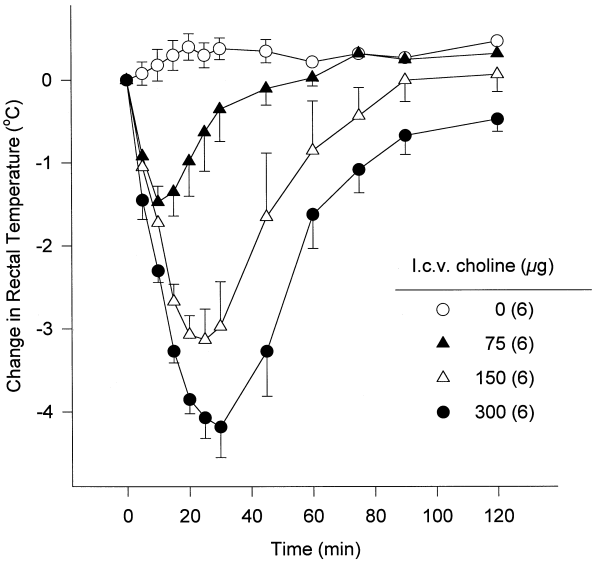

Fig. 1. Intracerebroventricular (i.c.v.) choline produces dose-dependent hypothermia. Freely-moving rats were treated with choline (75, 150 and 300 μg; i.c.v.). Data represent the means ± S.E. of the change in rectal body temperature of rats and were analyzed by repeated measures analysis of variance. The numbers in parentheses indicate the number of animals in each treatment group. All choline-treated groups differ significantly from saline-treated controls ($P < 0.01$). The baseline values at time 0 are: saline, 37.2 ± 0.2°C; 75 μg choline, 37.4 ± 0.1°C; 150 μg choline, 37.4 ± 0.2°C; 300 μg choline, 37.8 ± 0.1°C.

and choline has predominant effects on cholinergic neurotransmission. We found that the acetylcholine precursor, choline, produces profound dose-related hypothermia when injected centrally and that this effect is mediated mainly by muscarinic receptors.

2. Methods

Female Wistar rats (200–300 g; Experimental Animals Breeding and Research Center, Uludag University, Medical Faculty, Bursa, Turkey) were housed under a 12:12 h light:dark cycle with free access to food and water. Under light ether anesthesia, a 20-gauge stainless steel guide cannula was implanted in the right lateral ventricle 1.5 mm lateral to the midline, 1.0 mm posterior to the bregma and 4.0 mm below the skull surface. All experiments were performed 3–4 h after surgery between 1400 and 1600 h.

The drugs were dissolved in 10 μl isotonic saline and injected through a 26-gauge stainless steel cannula connected by PE-20 tubing to a 50-μl Hamilton syringe. The tip of the injection cannula was extended 0.5 mm below the end of the guide cannula and the injection volume was monitored by observing the movement of an air bubble placed in the tubing. Temperature measurements were recorded by inserting the thermistor probe of a thermometer (Ellab, Copenhagen, Denmark; accuracy 0.1°C) 6 cm into the colon. Experiments were performed at three ambient temperatures (T_a), 20–22°C, 4–6°C, and 30–32°C. Prior to each experiment, the animals were kept for 30 min at the appropriate T_a. The estrus stage of the rats was not determined, however, dose–response measurements and experiments with antagonists were conducted throughout the year and did not show variation.

The following drugs were used: choline chloride, atropine sulphate, mecamylamine hydrochloride, hemicholinium-3, pirenzepine dihydrochloride (Sigma, St. Louis, MO, USA), (11-[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1, 4]benzodiazepin-6-one) (AF-DX116) (Dr. Karl Thomae, Germany), hexahydro-siladifenidol hydrochloride (HHSD) (Research Biochemical, Natick, MA, USA). The drugs were dissolved in saline (0.9% NaCl).

Data are reported as the arithmetic means ± S.E. Statistical differences between treatment groups were determined by repeated measures analysis of variance performed with Systat (Version 5.03; Systat, Evaston, IL, USA).

3. Results

3.1. Intracerebroventricular choline produces dose-dependent hypothermia

Choline rapidly lowered the rectal temperature of rats when injected centrally. The choline-induced rectal tem-

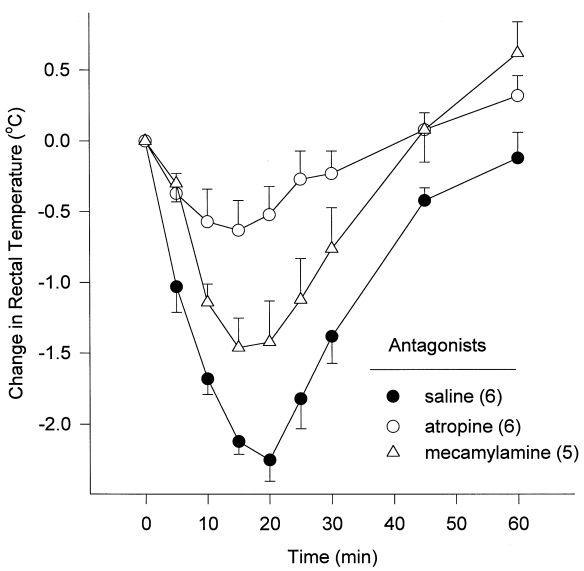

Fig. 2. The muscarinic antagonist, atropine, blocked choline-induced hypothermia whereas the nicotinic antagonist, mecamylamine, was only partially effective. Conscious rats were pre-treated with saline, atropine (10 μg; i.c.v.) or mecamylamine (20 μg; i.c.v.) and 20 min later, choline (150 μg, i.c.v.) was administered. The numbers in parentheses indicate the number of animals in each group. Data represent the mean ± S.E. rectal body temperature of rats and were analyzed by repeated measures analysis of variance. The atropine-pre-treated group differs significantly from saline treated controls ($P < 0.01$). The baseline values at time 0 are: saline, 37.6 ± 0.2°C; atropine, 37.5 ± 0.1°C; mecamylamine, 37.8 ± 0.2°C.

perature fall was dose-dependent, with decreases 1.5 ± 0.2, 3.1 ± 0.4 and 4.2 ± 0.4°C with doses of 75, 150 or 300 μg, respectively. Body temperature did not return to baseline values for 60 min after choline administration (150 or 300 μg) (Fig. 1).

Peripheral choline (30, 60 and 120 mg/kg; i.p.) injections did not produce hypothermia compared to the effect of saline (Fig. 6).

3.2. Muscarinic receptor antagonists block choline-induced hypothermia

Rats were pre-treated with saline, atropine or mecamylamine 20 min prior to choline injection (150 μg, i.c.v.). In the control group, choline injection after saline produced a 2.3 ± 0.2°C fall in body temperature. Pre-treatment with atropine (10 μg, i.c.v.), a non-selective muscarinic receptor antagonist, entirely blocked choline-induced hypothermia, whereas mecamylamine, a nicotinic receptor antagonist, (20 μg, i.c.v.) was only partially effective (Fig. 2). To further define the muscarinic receptor subtypes which mediate the hypothermic effect of choline, we pre-treated rats with either pirenzepine, AF-DX116 or HHSD to block muscarinic M_1, M_2 or M_3 receptors, respectively. We tested two doses of each antagonist (25 or 50 μg pirenzepine, 10 or 20 μg AF-DX116 and 50 or 100 μg HHSD). The doses were based on results of dose–response studies of antagonist effects in carbachol-induced hyperglycemia (Gurun and Ulus, unpublished data), but only the most effective dose for each antagonist is presented. We found that pirenzepine (25 μg, i.c.v.) and HHSD (100 μg, i.c.v.) blocked choline-induced hypothermia. Unlike the other antagonists, AF-DX116 produced a 2.1 ± 0.1°C ($n = 5$) fall in body temperature within 20 min when injected alone (10 μg, i.c.v.) (not shown). Pre-treatment with AF-DX116 at a higher dose (20 μg, i.c.v.), however, did not itself affect body temperature and did not inhibit choline-induced hypothermia (Fig. 3).

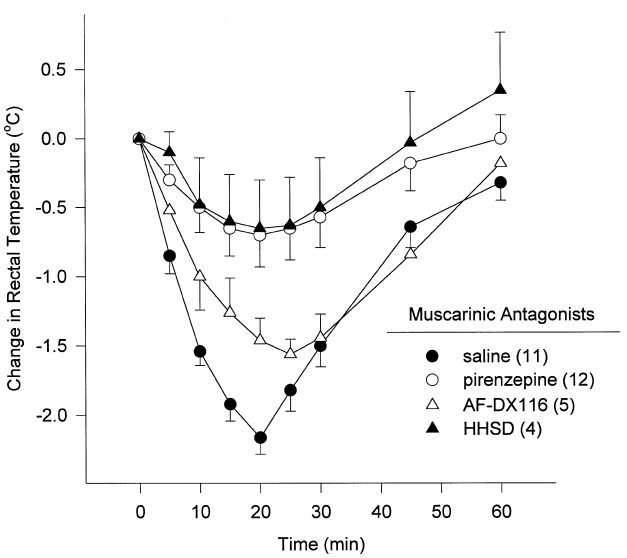

Fig. 3. M_1 and M_3, but not M_2 muscarinic receptor antagonists block choline-induced hypothermia. Rats were pre-treated with either saline, pirenzepine (25 μg, i.c.v.), AF-DX116 (20 μg, i.c.v.) or HHSD (100 μg, i.c.v.) 20 min prior to choline (150 μg, i.c.v.) injection. Data represent the mean ± S.E. body temperature of rats and were analyzed by repeated measures analysis of variance. The numbers in parentheses indicate the number of animals in each treatment group. Pirenzepine- and HHSD-pretreated groups differ significantly from saline-treated controls ($P < 0.01$). The baseline values at time 0 are: saline, 38.3 ± 0.2°C; pirenzepine, 37.7 ± 0.1°C; AF-DX116, 37.1 ± 0.4°C; HHSD, 37.8 ± 0.5.

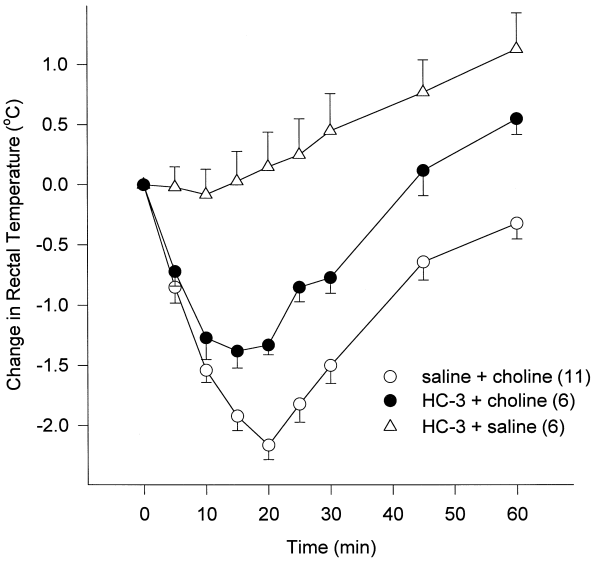

Fig. 4. Hemicholinium-3 attenuated choline-induced hypothermia. Rats were pre-treated with either saline or hemicholinium-3 (10 μg, i.c.v.) and 20 min later, saline-pre-treated rats received only choline, whereas hemicholinium-3-pre-treated rats received either saline or choline. The numbers in parentheses indicate the number of animals in each treatment group. Data represent the mean ± S.E. rectal body temperature. The baseline values at time 0 are: saline–saline, 38.3 ± 0.2°C, hemicholinium–saline, 36.6 ± 0.2°C: hemicholinium–choline, 36.4 ± 0.2°C.

3.3. Hemicholinium-3 attenuated choline-induced hypothermia

Hemicholinium-3 injection (5 μg) decreased body temperature 1.3 ± 0.2°C from baseline (37.8 ± 0.1°C) within 20 min. Choline (150 μg) or saline injection 20 min after hemicholinium-3 pre-treatment, produced an additional 1.3 ± 0.1°C or 0.1 ± 0.2°C decrease, respectively (Fig. 4). Compared to saline pre-treatment that lowered the body temperature 2.2 ± 0.1°C, hemicholinium-3 pre-treatment attenuated the choline-induced hypothermia by 36%.

3.4. The effect of ambient temperature on choline-induced hypothermia

Central choline injections (0–300 μg) at T_a 4–6°C, produced a reduction in body temperature similar to that at T_a 20–22°C (Fig. 5). On the other hand, choline's hy-

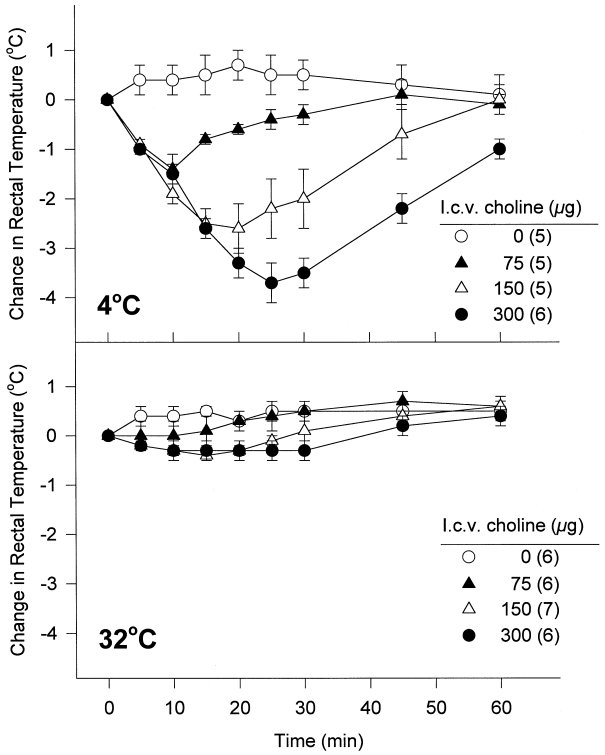

Fig. 5. The effects of T_a on choline-induced hypothermia. Prior to experiments, the animals were maintained for 30 min at the appropriate T_a. At 4–6°C, choline produced dose-dependent (0–300 μg, i.c.v.) hypothermia whereas at 30–32°C it did not induce hypothermia. The baseline values at time 0 for 4–6°C T_a: saline 38.0±0.3°C, 75 μg choline, 38.5±0.2°C; 150 μg choline, 38.4±0.1°C; 300 μg choline, 38.8±0.1°C. The baseline values for 30–32°C T_a: saline, 37.9±0.1°C, 75 μg choline, 37.4±0.2°C; 150 μg choline, 37.8±0.1°C; 300 μg choline, 37.7±0.1°C.

pothermic effect was totally blocked by a shift of T_a to 30–32°C (Fig. 5).

4. Discussion

Choline is the precursor of the cholinergic neurotransmitter, acetylcholine, yet its pharmacological effects have not been widely investigated. Here, we report that like other cholinergic drugs, choline produces dose-dependent hypothermia mediated primarily by muscarinic receptors (Fig. 1) when injected centrally but not peripherally.

In earlier studies, the muscarinic receptor antagonist, atropine, was found to be very effective to block the hypothermia induced by acetylcholine and other cholinergic agents when injected centrally (Lomax and Jenden, 1966; Lin et al., 1980). Similarly, we found that atropine entirely blocked choline-induced hypothermia, but mecamylamine, a nicotinic receptor antagonist, showed a very limited effect (Fig. 2). These findings suggest that choline-induced hypothermia is mainly mediated by muscarinic receptors. Nicotinic receptors might also play role in choline-induced hypothermia but to a lesser extent.

The existence of central muscarinic receptor subtypes has been demonstrated (Hulme et al., 1990). Receptor binding experiments have shown at least three different muscarinic receptors and results of in vivo studies with sympathetic ganglia, heart and salivary glands support the existence of these subtypes (Doods et al., 1987). Sánches and Lembøl (1994) showed that muscarinic receptor subtypes play different roles in the mediation of hypothermia, tremor and salivation. In their study, oxotremorine-induced hypothermia was blocked by pirenzepine and 4-DAMP (4-diphenylacetoxy-*N*-methylpiperidine) methiodide (muscarinic M_3 receptor antagonist), but not by AF-DX116. Consistent with their findings, we found that choline-induced hypothermia is also mediated by muscarinic M_1 and M_3, receptors since pirenzepine and HHSD blocked choline-induced hypothermia. In contrast, the muscarinic M_2 receptor antagonist, AF-DX116, did not affect choline-induced hypothermia (Fig. 3) when 20 μg (i.c.v.) was injected, however, it produced hypothermia itself at a lower dose (10 μg) (not shown). Since the selective blockade of muscarinic M_2 receptors was shown to produce both biochemical and behavioral signs of acetylcholine release (Hoss et al., 1990), AF-DX116's hypothermic effect might be attributable to an increased acetylcholine release.

To define whether choline-induced hypothermia is mediated by increased acetylcholine synthesis and release and/or by choline's direct action on muscarinic receptors, we pre-treated rats with hemicholinium-3, a high-affinity choline uptake inhibitor and found that it slightly attenu-

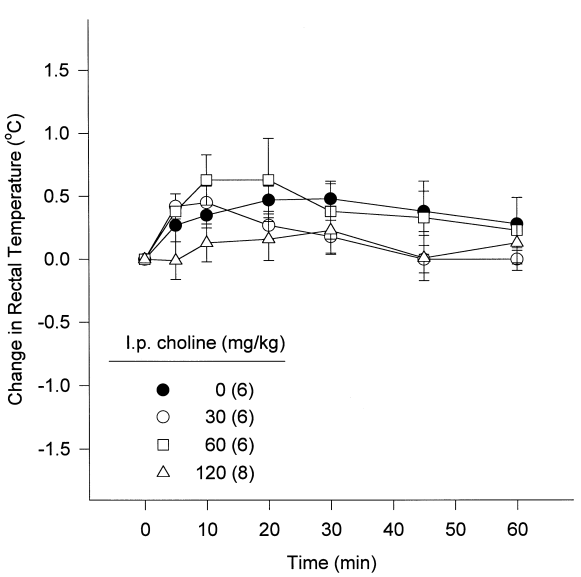

Fig. 6. Peripheral effects of choline on body temperature. Choline (30, 60 or 120 mg/kg, i.p.) or saline was administered to freely moving rats. Peripheral choline injections did not produce an effect on body temperature. The baseline values at time 0 are: saline, 37.6±0.2°C; 30 mg/kg choline, 37.5±0.2°C; 60 mg/kg choline, 37.5±0.1°C; 120 mg/kg choline, 37.4±0.2°C.

ated choline-induced hypothermia (Fig. 4). In the light of these data, it is conceivable that choline's hypothermic effect is at least partially mediated by choline-induced acetylcholine synthesis and release. However, as reported before (Lin et al., 1980), hemicholinium-3 produced its own hypothermic effect during pre-treatment in our experiments (1.3 ± 0.2°C) and therefore, we were unable to find whether choline's effect can be attributable to increased acetylcholine synthesis and release. The mechanism of hemicholinium-3 hypothermia is controversial, but results of recent in vitro studies indicate that hemicholinium-3 might increase acetylcholine release to some extent by activating presynaptic nicotinic receptors (Poulain et al., 1987; Umeda and Sumi, 1990). It is likely that choline produces some hypothermia by acting directly at acetylcholine receptors and hemicholinium-3-resistant choline-induced hypothermia might also be mediated by this mechanism (Ulus et al., 1988).

Previously, it was shown that choline increases pilocarpine-induced hypothermia when injected intraperitoneally (Pomara et al., 1983). Pomara suggested, based on receptor binding experiments (Palacios and Kuhar, 1979; Speth and Yamamura, 1979), that choline's hypothermic effect is mediated by a direct action on muscarinic receptors. However, recent in vivo brain dialysis experiments arguing against this hypothesis showed that i.p. choline administration caused a negligible increase in extracellular choline levels compared to the acetylcholine release in cholinergic brain regions (Johnson et al., 1992; Buyukuysal et al., 1995). In the light of these experiments, we injected choline intraperitoneally (30, 60 and 120 mg/kg, i.p.) and found that choline did not affect body temperature at any of the doses tested (Fig. 6). Since peripheral choline injections (120 mg/kg, i.p.) are known to elevate brain acetylcholine levels, the ineffectiveness of choline to induce hypothermia might be a result of the choline concentration not reaching the necessary level, or of peripheral effects of choline counteracting the central effects.

Finally, we tested whether choline-induced hypothermia is affected by ambient temperature changes. We found that choline produces hypothermia at 4–6°C, but that this effect was abolished by a shift of the ambient temperature to 30–32°C (Fig. 5). This finding is also consistent with results of previous work showing that the hypothermia induced by cholinergic agents is affected by ambient temperature (Lin et al., 1980).

In summary, choline, similarly to acetylcholine, produces hypothermia when injected centrally and this effect is primarily mediated by muscarinic M_1 and M_3 receptors. We believe better characterization of choline hypothermia might contribute to the drug development efforts for the treatment of brain ischemia with choline-containing drugs. Since hypothermia is known to be neuroprotective, the effectiveness of choline-containing drugs as neuroprotective agents should be evaluated in view of their hypothermic effect.

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