Cobaltous Chloride-Induced Hypothermia in Mice III: Effect of Pretreatment with 5-Hydroxytryptaminergic Agents

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Received June 10, 1982, from the *Divisions of Pharmacology and Physiology, Marquette University School of Dentistry, Milwaukee, WI 53233.* Accepted for publication July 1, 1982.

Abstract □ The influence of various 5-hydroxytryptaminergic agonist and antagonist drugs on body-temperature response to cobaltous chloride in mice was noted. Pretreatment with *p*-chloroamphetamine, *p*-chlorophenylalanine, and *p*-iodoamphetamine antagonized the body-temperature response to cobalt. *p*-Chloroamphetamine and *p*-chlorophenylalanine reduced, while *p*-iodoamphetamine elevated, brain serotonin levels. The uptake inhibitor agents, fluoxetine and nisoxetine, failed to modify the ability of *p*-chloroamphetamine to antagonize cobalt hypothermia. Cyproheptadine, methergoline, and xylamidine pretreatment enhanced rather than antagonized body-temperature depression by cobalt. Tryptophan hydroxylase inhibitors antagonized cobalt hypothermia, but receptor-blocking agents were without influence, indicating that antagonism was mediated through mechanisms other than the depletion of serotonin. Elevation rather than depletion of brain serotonin by *p*-iodoamphetamine and failure of uptake inhibitors to modify *p*-chloroamphetamine antagonism of cobalt hypothermia lend further support for a nonserotonergic role of these amines in their ability to antagonize body-temperature depression by cobaltous chloride in mice.

Keyphrases □ Cobaltous chloride—induced hypothermia in mice, pretreatment with 5-hydroxytryptaminergic agents □ Hypothermia—induction in mice with cobaltous chloride, effects of pretreatment with 5-hydroxytryptamingeric agents

Cobaltous chloride has been reported to produce a dose-dependent depression of body temperature in several species, apparently through a centrally mediated decrease in heat production (1). Subsequent studies in this laboratory revealed that chlorpromazine was capable of antagonizing cobalt-induced hypothermia in mice, and it was concluded that central α-receptor blockade and/or peripheral antihistaminic activity, but not anticholinergic activity, may have contributed to this partial antagonism (2). Most recently, *p*-chloroamphetamine hydrochloride has been shown to attenuate markedly the body temperature response to cobalt in this same species (approximately 60% antagonism) (3). Although this compound has not been extensively studied in mice, it appeared possible that its well-known antiserotonin qualities may contribute to antagonism of the body temperature depression. The present report describes the influence of various 5-hydroxytryptaminergic agonists and antagonists on the body-temperature response to cobaltous chloride in mice.

EXPERIMENTAL

Male Swiss albino mice (20–30 g) were used in this investigation. The animals were housed in groups of 6–20 with *ab libitum* access to food[1] and water for at least 3 days prior to use. The mice were kept in a draft-free room under constant-temperature conditions (23 ± 1°) and maintained on a 12-hr light/dark cycle. All drugs were freshly prepared with distilled water in concentrations (calculated as the salt) such that a volume of 0.01 ml/g was delivered. Methergoline was solubilized with distilled water acidified to pH 3.8 with ascorbic acid. All injections were delivered by the intraperitoneal route.

A thermistor thermometer[2] was used for obtaining rectal temperature. Temperatures were recorded with a thermistor probe inserted 2.5 cm and held in position until constant readings were attained.

At the beginning of each experiment the mice were placed in individual circular wire-mesh cages, and weights were obtained with a triple-beam balance[3], after which initial temperatures were recorded and the treatment was administered. Temperatures were recorded again at various intervals. Control animals received distilled water (0.01 ml/g). To study the influence of various serotonergic agonist and antagonist agents on cobalt-induced hypothermia, pretreatment injections (water, *p*-chloroamphetamine HCl[4], *p*-iodoamphetamine HCl[5], *p*-chlorophenylalanine methyl ester HCl[4], fluoxetine HCl[5], nisoxetine HCl[5], cyproheptadine HCl[6], methergoline[7], and xylamidine tosylate[8]) were given at specified intervals prior to recording the initial temperatures.

Serotonin levels were recorded with a spectrophotofluorometer[9] according to the method of Curzon and Green (4), modified by Hyyppä *et al.* (5), with a standard curve relating relative fluorescence to serotonin concentration.

To compare mean temperature changes (*i.e.*, the difference between temperature immediately prior to and at the appropriate interval following treatment), statistical significance was determined by use of Student's *t* test. Temperature differences were considered significant at the probability level of 5% or less.

RESULTS

Cobaltous chloride (25 mg/kg ip) was administered at the appropriate interval following pretreatment with *p*-chloroamphetamine, *p*-iodoamphetamine, and *p*-chlorophenylalanine in groups of at least eight animals; body temperatures were monitored for a 4-hr period. The results of this experiment are shown in Fig. 1. Pretreatment with a single daily dose of 100 mg/kg of *p*-chlorophenylalanine for 3 consecutive days prior to the intraperatoneal administration of cobaltous chloride resulted in a reduction in the intensity and duration of the body temperature response. This antagonism was similar, but of less magnitude, to that seem in the case of *p*-chloroamphetamine (10 kg/kg ip 1 hr prior to cobalt). *p*-Iodoamphetamine pretreatment (10 mg/kg ip 1 hr before cobalt) also antagonized cobalt hypothermia and was less potent than *p*-chloroamphetamine.

Whole-brain serotonin levels were determined 2 hr following administration of *p*-chloroamphetamine and *p*-iodoamphetamine and 24 hr following the last of three single daily doses of 100 mg/kg of *p*-chlorophenylalanine in mice. *p*-Chloroamphetamine and *p*-chlorophenylalanine both significantly reduced whole-brain serotonin levels, while *p*-iodoamphetamine increased serotonin levels at the 2-hr interval (Table I).

The influence of fluoxetine and nisoxetine on the ability of *p*-chloroamphetamine to antagonize cobalt body temperature depression is presented in Table II. These uptake inhibitor agents both failed to modify the *p*-chloroamphetamine antagonism in doses known to reduce *p*-chloroamphetamine-induced serotonin depletion.

The body-temperature response to cobaltous chloride (25 mg/kg ip) was monitored at various intervals following the administration of 1 mg/kg each of cyproheptadine, methergoline, and xylamidine. In every

[1] Wayne Lab Blox.

[2] Model 46 Tele-thermometer, Yellow Springs Instrument Co., Yellow Springs, Ohio.
[3] Ohaus.
[4] Sigma Chemical Co.
[5] Eli Lilly and Co. Research Laboratories.
[6] Merck Sharp and Dohme Laboratories.
[7] Framitalia.
[8] Wellcome Research Laboratories.
[9] Aminco-Bowman.

0022-3549/83/0700-0824$01.00/0

Table I—Effect of Intraperitoneal *p*-Chloroamphetamine, *p*-Chlorophenylalanine, and *p*-Iodoamphetamine on Serotonin Concentration in Mice Brains

Drug	Serotonin, μg/g ± *SE*[a]		
	Controls	Treated[b]	Change, %
p-Chloroamphetamine	0.6585 ± 0.012	0.4413 ± 0.015	−32.98
p-Chlorophenylalanine	0.6612 ± 0.025	0.5904 ± 0.020	−10.71
p-Iodoamphetamine	0.6549 ± 0.022	0.7208 ± 0.021	+10.06

[a] Brains were removed from groups of at least eight mice 2 hr following the injection of 10 mg/kg of *p*-chloroamphetamine and *p*-iodoamphetamine and 24 hr following the third of three consecutive daily injections of 100 mg/kg of *p*-chlorophenylalanine. Control animals received 0.01 ml/g body weight of water instead of drug treatment. [b] Compared with appropriate water-treated control, $p < 0.05$.

case, 5-hydroxytryptaminergic receptor-blocking agent pretreatment enhanced rather than antagonized body temperature depression (Table III). The ascorbic acid vehicle for solubilizing methergoline did not alter body temperature, nor did it influence the body temperature response to cobaltous chloride.

DISCUSSION

The nature of body temperature response to cobaltous chloride in mice at present is poorly understood. However, the recent observation that cobalt hypothermia could be antagonized in part by pretreatment with *p*-chloroamphetamine suggests a possible serotonergic involvement (3). For example, cobalt may have acted indirectly in the CNS to release serotonin from neuronal storage sites into the vicinity of postsynaptic serotonergic receptors involved in the regulation of body temperature. One problem, however, with the use of *p*-chloroamphetamine as a pharmacological tool in the short term is its lack of specificity. For example, in addition to depletion of serotonin, this halogenated arylalkylamine has been shown to alter dopamine and norepinephrine synthesis in the rat (6). Thus, it is conceivable that other (nonserotonergic) mechanisms might also contribute to the development of hypothermia following the administration of cobaltous chloride.

The influence of *p*-chloroamphetamine on serotonergic nerve function has been extensively studied in the rat. Pletscher *et al.* (7) first reported that this compound causes a marked reduction in brain concentrations of 5-HT and 5-hydroxyindoleacetic acid in this species. Several mechanisms have been proposed to explain the action of this agent at the cellular level. Of these mechanisms, the inhibition of the rate-limiting enzyme tryptophan hydroxylase might possibly account for the rapid depletion of serotonin by *p*-chloroamphetamine in the short term. *p*-Iodoamphetamine also has been reported to decrease serotonin synthesis through the inhibition of tryptophan hydroxylase (8). One of the most commonly employed tryptophan hydroxylase inhibitors is *p*-chlorophenylalanine (9). The present investigation has shown that all three tryptophan hydroxylase inhibitors were capable of reducing the intensity and duration of cobalt hypothermia. These findings suggest that antagonism was accomplished through serotonin depletion, perhaps through inhibition of the rate-limiting enzyme in the synthesis of serotonin. However, similar antagonism of cobalt-induced hypothermia with central or peripheral serotonin receptor-blocking agents could not be demonstrated. The latter observations do not support the suggestion that *p*-chloroamphetamine antagonism of cobalt hypothermia was mediated through an antiserotonin influence.

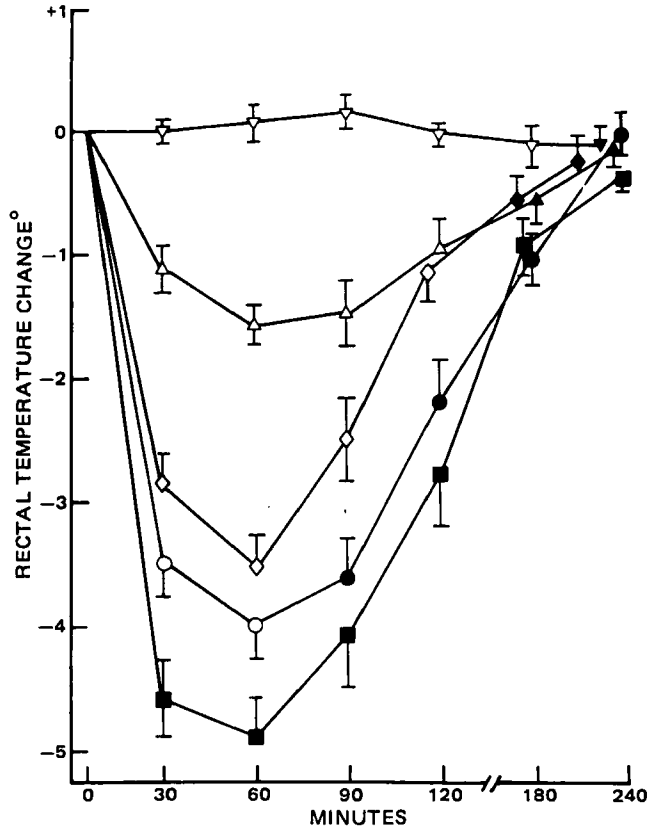

Figure 1—*Time course of the effect of 0.01 ml/g ip of distilled water (-▼-); 25 mg/kg ip of cobaltous chloride (-■-); 10 mg/kg ip of* p-*chloroamphetamine hydrochloride 1 hr prior to 25 mg/kg ip of cobaltous chloride (-▲-); three consecutive daily doses of 100 mg/kg ip of* p-*chlorophenylalanine methyl ester hydrochloride 24 hr prior to 25 mg/kg ip of cobaltous chloride (-●-); 10 mg/kg ip of* p-*iodoamphetamine 1 hr prior to 25 mg/kg ip of cobaltous chloride (-◆-) on the rectal temperature in mice. Water and cobaltous chloride were administered at time zero. Open symbols denote significant difference ($p < 0.05$) from cobalt treatment at the corresponding time interval. Each point represents the average of at least eight determinations. Vertical bars represent standard errors.*

Little information exists in the literature with respect to halogenated amphetamine effects on serotonergic neuronal function in mice, particularly in the case of *p*-iodoamphetamine. A previous study showed that *p*-chloroamphetamine causes depletion of serotonin from the brains of mice (3). In the present study we were unable to demonstrate a similar depletion with *p*-iodoamphetamine. In contrast, serotonin levels were significantly increased at the 2-hr interval following the intraperitoneal injection of *p*-iodoamphetamine. These observations suggest that mechanisms other than depletion of serotonin are involved in antagonism of cobalt-induced hypothermia by the halogenated arlalkylamines, *p*-

Table II—Interaction of Fluoxetine and Nisoxetine with *p*-Chloroamphetamine on the Body Temperature Response to Cobaltous Chloride in Mice

Pretreatment[a]	Treatment[b]			
	Water		Cobaltous Chloride	
	Initial Temperature °[c]	Temperature Change °[d] (Mean ± *SE*)	Initial Temperature °[c]	Temperature Change °[d] (Mean ± *SE*)
Water–Water	36.82	+0.10 ± 0.09	36.88	−4.38 ± 0.24[e]
Water–*p*-Chloroamphetamine	37.04	+0.04 ± 0.13	36.82	−1.13 ± 0.19[f]
Fluoxetine–Water	36.95	+0.29 ± 0.21	36.90	−4.79 ± 0.47
Fluoxetine –*p*-Chloroamphetamine	36.74	−0.07 ± 0.14	36.89	−1.29 ± 0.28[f]
Nisoxetine–Water	37.07	+0.03 ± 0.27	37.16	−4.62 ± 0.40
Nisoxetine–*p*-Chloroamphetamine	37.03	+0.01 ± 0.27	36.97	−0.73 ± 0.51[f]

[a] Fluoxetine hydrochloride (10 mg/kg ip), nisoxetine hydrochloride (10 mg/kg ip), or water (0.01 ml/g ip) were administered 2 hr prior to *p*-chloroamphetamine hydrochloride (10 mg/kg ip) or water (0.01 ml/g ip) to groups of 10 mice. [b] Intraperitoneal water (0.01 ml/g) and cobaltous chloride (25 mg/kg) treatments were given 1 hr following the final pretreatment injection. [c] Initial temperatures were recorded immediately prior to water or cobalt treatment. [d] Temperature changes represent the difference between body temperature recorded initially and that obtained 30 min after treatment. [e] Compared with water–water (pretreatment) water (treatment), $p < 0.05$. [f] Compared with water–water (pretreatment) cobalt (treatment), $p < 0.05$.

Table III—Effect of Pretreatment with 5-Hydroxytryptaminergic Receptor Blocking Agents on the Body Temperature Response to Cobaltous Chloride in Mice

Pretreatment[a]	Treatment[b]			
	Water		Cobaltous Chloride	
	Initial Temperature °[c]	Temperature Change °[d] (Mean ± SE)	Initial Temperature °[c]	Temperature Change °[d] (Mean ± SE)
Water	36.83	+0.13 ± 0.09	36.96	−4.73 ± 0.33[e]
Methergoline[f]	36.83	+0.25 ± 0.14	37.02	−6.12 ± 0.47[g]
Cyproheptadine	37.28	+0.03 ± 0.23	37.22	−5.97 ± 0.48[g]
Xylamadine	36.85	+0.22 ± 0.13	36.74	−5.56 ± 0.34[g]

[a] Water (0.01 ml/g), methergoline (1 mg/kg), cyproheptadine hydrochloride (1 mg/kg), and xylamidine tosylate (1 mg/kg) were administered intraperitoneally to groups of 10 animals. [b] Intraperitoneal water (0.01 ml/g) and cobaltous chloride (25 mg/kg) treatments were given 4 hr following pretreatment injection. [c] Initial temperatures were recorded immediately prior to water or cobalt treatment. [d] Temperature changes represent the difference between body temperature recorded initially and that obtained 30 min after treatment. [e] Compared with water–water (Pretreatment–Treatment), $p < 0.05$. [f] Methergoline was solubilized in distilled water acidified to pH 3.8 with ascorbic acid. [g] Compared with water–cobalt (Pretreatment–Treatment), $p < 0.05$.

chloroamphetamine and *p*-iodoamphetamine. In addition, the finding that uptake inhibitors (fluoxetine and nisoxetine) failed to modify halogenated amphetamine reversal of cobalt hypothermia further supports an extraserotonergic role of these amines in their ability to antagonize body temperature depression by cobaltous chloride.

The results presented in this report do not support the suggestion that cobaltous chloride hypothermia is mediated through the release of serotonin from intraneuronal storage sites. For example, the serotonin receptor-blocking agents, cyproheptadine, methergoline, and xylamidine, were incapable of attenuating the body temperature response. While it seems inviting to postulate an influence of cobalt on the neuronal storage of dopamine and/or norepinephrine, previous studies utilizing 6-hydroxydopamine failed to reveal catecholaminergic involvement (2). It is likely that cobalt produces hypothermia through a mechanism or mechanisms which are at present undefined.

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ACKNOWLEDGMENTS

Presented at the Pharmacology and Toxicology Section, APhA Academy of Pharmaceutical Sciences Meeting, Orlando, Florida, November 1981.

Supported in part by National Institutes of Health Grant RR-09016.

The authors are indebted to Laura Huspen and Deborah Redford for assistance, and to Mary Jonas Owen for typing this manuscript. The authors thank Dr. R. W. Fuller of Eli Lilly and Company and Dr. P. J. McHale of the Wellcome Research Laboratories for their generosity in supplying *p*-iodoamphetamine and xylamidine.

Effect of Cimetidine on the Pharmacokinetics of Quinidine and Lidocaine in the Rat

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Received March 23, 1982, from the *Department of Pharmacology, Hahnemann Medical College and Hospital, Philadelphia, PA 19102.* Accepted for publication July 9, 1982.

Abstract □ Because of previously reported drug interactions involving cimetidine and liver-metabolized drugs, the intravenous pharmacokinetics of quinidine (25 mg/kg) and lidocaine (15 mg/kg) were investigated in anesthetized rats pretreated with a single intraperitoneal dose of cimetidine (60 mg/kg) and compared with saline pretreated controls. Significant reductions of 35 and 23% in the respective total clearances of quinidine and lidocaine were observed in the presence of cimetidine. The quinidine volume of distribution was significantly decreased in the cimetidine-treated rats, while the lidocaine volume of distribution was not altered significantly. There was no significant change in the elimination half-life for either drug in the presence of cimetidine. These results suggest cautious use of quinidine or lidocaine when cimetidine is prescribed concurrently.

Keyphrases □ Cimetidine—inhibition of lidocaine and quinidine clearances in the rat, kinetics □ Lidocaine—inhibition of clearance in the rat by cimetidine, kinetics □ Quinidine—inhibition of clearance in the rat by cimetidine, kinetics □ Kinetics—of the inhibition of lidocaine and quinidine clearances in the rat by cimetidine

Cimetidine, a histamine H_2-receptor antagonist, is prescribed widely for the therapy of peptic ulcers. Human pharmacokinetic studies have demonstrated that cimetidine in therapeutic doses impairs the elimination of drugs metabolized by cytochrome P-450-dependent pathways, such as antipyrine (1), theophylline (1), diazepam (2),

0022-3549/83/0700-0826$01.00/0