Cobaltous Chloride-Induced Hypothermia in Mice I: Effect of Pretreatment with Anticholinergic Drugs

DAVID H. BURKE

Received September 1, 1977, from the *Division of Pharmacology, School of Dentistry, Marquette University, Milwaukee, WI 53233.*
Accepted for publication September 26, 1977.

Abstract □ The hypothermic response following intraperitoneal doses (6.25, 12.5, and 25 mg/kg) of cobaltous chloride was investigated in Swiss albino mice. The magnitude and duration of rectal temperature depression were dose related. In each case, maximal hypothermia was evident within 30 min after injection. Body temperature depression was noted 30 min after oral, subcutaneous, intraperitoneal, intravenous, and intracerebral administration of cobaltous chloride. Cobalt was most active when administered intracerebrally, suggesting a central component to the thermolytic response. Rectal temperature depression following cobaltous chloride was dependent on the ambient temperature. The time course of the effect of cobaltous chloride on rectal and cutaneous tail temperature was noted. Cutaneous tail temperature depression occurred throughout the rectal temperature response, suggesting that cobalt may decrease heat production. Pretreatment with atropine sulfate, hexamethonium bromide, or nicotine failed to modify the temperature response to cobalt. Chlorpromazine hydrochloride pretreatment resulted in a partial antagonism of cobalt-induced hypothermia, presumably through a mechanism other than cholinergic blockade.

Keyphrases □ Cobaltous chloride—mechanism of hypothermic response in mice, effect of pretreatment with anticholinergics □ Hypothermia—induced by cobaltous chloride in mice, mechanism, effect of pretreatment with anticholinergics □ Anticholinergics—effect of pretreatment on hypothermic response to cobaltous chloride in mice

The complex system that maintains body temperature within narrow limits in homiotherms is highly susceptible to insult by drugs and chemicals. For example, the general anesthetics and peripheral vasodilators cause hypothermia through a nonspecific depression of the central nervous system (CNS) (1). Nitroprusside produces hypothermia indirectly through the influence of released cyanide on cellular respiration (2). Oxotremorine causes hypothermia through a central cholinergic mechanism (3, 4).

Pilot studies in this laboratory revealed that cobaltous chloride produces a pronounced fall in the body temperature of mice. This study investigated the temperature response to cobaltous chloride in mice. It was anticipated that the findings would provide insight into the mechanism by which this agent produces body temperature depression.

EXPERIMENTAL

Male Swiss albino mice, 20–25 g, were housed in groups of 20 with *ad libitum* access to laboratory food[1] and water for several days prior to the study. For 24 hr prior to and including the experiment time, the mice were kept in a draft-free room at a constant temperature.

Drug solutions were freshly prepared with distilled water in concentrations (calculated as the salt) such that a volume of 0.01 ml/g was delivered. Intracerebral injections, however, were administered in a fixed volume of 0.01 ml/mouse.

A thermistor thermometer[2] was used for obtaining rectal and cutaneous temperatures. Rectal temperatures were recorded with a thermistor probe inserted to a distance of 2.5 cm and held in position until constant readings were attained. Cutaneous temperatures were obtained by placement of a disk probe on the base of the tail. A small amount of electrode paste was applied to the surface of the disk probe.

Intracerebral injections were accomplished according to the method of Haley and McCormick (5). Cobaltous chloride or water (warmed to 38°) was administered at a point lateral to the midline joining the anterior bases of the ears. A 22-gauge needle attached to a microliter syringe[3] was inserted through the skull to a depth of 3 mm. The prior injection of several animals with a solution of methylene blue (0.5%) resulted in dye localization in the third and fourth ventricles.

At the start of each day's testing, the mice were placed singly in circular wire-mesh cages and individual weights were obtained with a triple-beam balance[4]. Immediately following weight determination, initial temperatures were recorded and treatment administration was accomplished. Temperatures were recorded again at various intervals. Unless otherwise stated, all treatments were given intraperitoneally. Controls received distilled water (0.01 ml/g). To study the influence of various anticholinergic agents on cobalt-induced hypothermia, pretreatment injections (water, atropine sulfate, chlorpromazine hydrochloride, hexamethonium bromide, or nicotine) were given 30 min prior to recording initial temperatures.

In comparison of 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 the Student *t* test. Temperature differences were considered significant at the probability level of 5% or less.

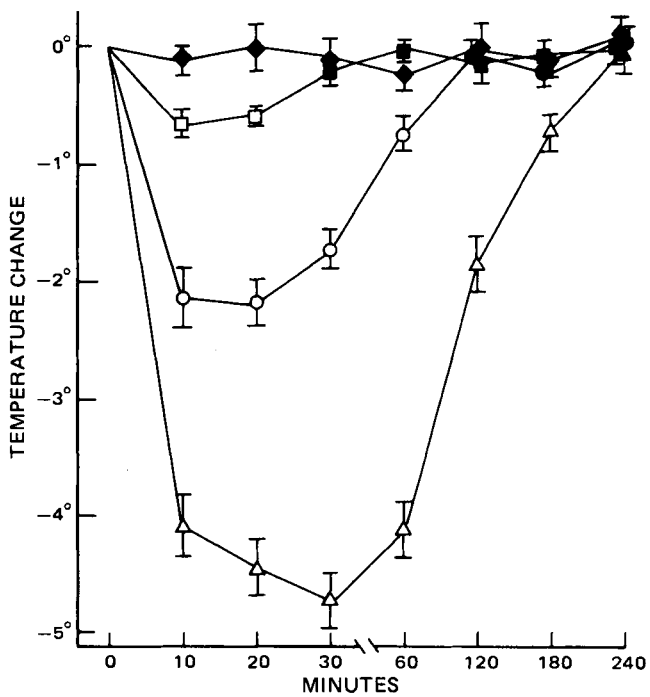

Figure 1—*Time course of the effect of a 0.01-ml/g ip dose of distilled water (◆) and 6.25- (■), 12.5- (●), and 25- (▲) mg/kg ip doses of cobaltous chloride on the rectal temperature in mice. Water and cobaltous chloride were administered at zero time. Open symbols denote significant difference ($p < 0.05$) from water treatment at the corresponding time interval. Each point represents the average of 12 determinations. Vertical bars represent standard errors.*

[1] Wayne Lab, Blox.
[2] Model 46 Tele-thermometer, Yellow Springs Instrument Co., Yellow Springs, Ohio.
[3] Hamilton.
[4] Ohaus.

Table I—Body Temperature Changes 30 min after Cobaltous Chloride Administration by Various Routes in Mice

Route	Water		Cobaltous Chloride (25 mg/kg)	
	Initial Temperature	Temperature Change (Mean ± *SE*)[a]	Initial Temperature	Temperature Change (Mean ± *SE*)[a,b]
Oral	37.94°	−0.03 ± 0.15°	38.06°	−2.18 ± 0.53°
Subcutaneous	37.53°	−0.34 ± 0.25°	37.68°	−2.72 ± 0.41°
Intraperitoneal	37.57°	−0.24 ± 0.16°	37.73°	−3.98 ± 0.17°
Intravenous	37.83°	+0.05 ± 0.19°	37.80°	−3.84 ± 0.29°
Intracerebral	37.93°	−0.68 ± 0.15°	38.03°	−4.38 ± 0.28[c]

[a] Temperature changes represent the difference between body temperature recorded initially and that obtained 30 min following water or cobaltous chloride treatment in groups of 12 mice. [b] Compared to water by the corresponding route, $p < 0.05$. [c] Each animal received 0.01 ml of a 0.25% cobaltous chloride solution.

Table II—Body Temperature Changes 30 min after Intraperitoneal Injection of Cobaltous Chloride to Mice at Various Ambient Temperatures

Ambient Temperature	Water		Cobaltous Chloride (25 mg/kg)	
	Initial Temperature	Temperature Change (Mean ± *SE*)[a]	Initial Temperature	Temperature Change (Mean ± *SE*)[a]
18°	36.49°	+0.25 ± 0.14°	36.32°	−6.33 ± 0.41°[b]
23°	37.33°	−0.04 ± 0.19°	37.93°	−4.11 ± 0.51°[b]
28°	37.68°	−0.21 ± 0.13°	38.07°	−1.07 ± 0.14°[b]
33°	38.47°	+0.16 ± 0.18°	38.93°	+0.28 ± 0.14°

[a] Temperature changes represent the difference between body temperature recorded initially and that obtained 30 min following water or cobaltous chloride treatment in groups of 12 mice. [b] Compared to water at the corresponding ambient temperature, $p < 0.05$.

RESULTS

The effect of cobaltous chloride (6.25, 12.5, and 25 mg/kg ip) on the rectal temperature of mice over 4 hr is shown in Fig. 1. At the dosage level of 6.25 mg/kg, cobalt produced body temperature depression lasting approximately 20 min. The magnitude and duration of the hypothermic response were increased with higher doses. Body temperature often fell by more than 6° within 30 min of injection of cobaltous chloride at the 25-mg/kg level. Hypothermia was always accompanied by depression of locomotor activity. Visible shivering and increased muscle tone were unapparent during and upon recovery from hypothermia.

Cobaltous chloride was administered to groups of 12 mice by several routes, and the effect on body temperature was noted 30 min later (Table I). Significant hypothermia occurred following administration of this agent (25 mg/kg) by the oral (−2.18°), subcutaneous (−2.72°), intraperitoneal (−3.98°), and intravenous (−3.84°) routes. Cobalt was most active when administered intracerebrally; fixed doses (0.01 ml of a 0.25% solution) caused an average temperature drop of 4.38°.

The influence of ambient temperature on the thermal response of mice to cobaltous chloride (25 mg/kg ip) is illustrated in Table II. Animals exposed to environmental temperatures of 18, 23, and 28° had respective drops of 6.33, 4.11, and 1.07°; mice exposed to 33° ambient temperature did not exhibit hypothermia.

Figure 2 depicts the time course of changes in rectal and cutaneous tail temperatures after the administration of cobaltous chloride (25 mg/kg ip). Immediately prior to drug injection, rectal and cutaneous temperatures averaged 37.67 and 25.41°, respectively. The rectal temperature response was similar to that presented in Fig. 1. Cutaneous temperature was depressed throughout the entire rectal temperature response.

The effect of pretreatment with various compounds on the hypothermic response to cobaltous chloride (25 mg/kg ip) is presented in Table III. Atropine (5.0 mg/kg), nicotine (0.25 mg/kg), and hexamethonium (10 mg/kg) were incapable of altering cobalt-induced body temperature depression. The combination of chlorpromazine (1.0 mg/kg) and cobaltous chloride resulted in hypothermia amounting to 2.36°. This value was significantly higher than the 4.33° drop obtained with cobalt in the presence of water.

DISCUSSION

A paucity of information exists concerning the influence of cobalt(II) on the mammalian body temperature. Hypothermia in rats was reported (6) following cobaltous chloride administration, 4 mg/kg. The results of the present investigation showed cobaltous chloride to be a potent hypothermic agent in mice. The intensity of this response was dependent on the dose, route of administration, and ambient temperature.

Evidence has been provided that cobalt elicits hypothermia through an action on the CNS. For example, in doses incapable of causing body temperature depression systemically, the intracerebral administration of this agent produced a marked hypothermic response.

Hypothermia results from either a decrease in heat production or an increase in heat dissipation. Cobaltous chloride appears to lower body temperature by decreasing heat production. For example, throughout the entire rectal temperature response, cutaneous tail temperature was reduced (Fig. 2).

Cobaltous chloride appears to produce a collapse of thermoregulation rather than a shift in the hypothalamic set point. Table II shows that mice exposed to an environmental temperature of 18° produced a mean fall in body temperature of 6.33°, as compared to a 1.07° drop following exposure to an ambient temperature of 28°. Exposure to a high environmental temperature (33°) prevented this hypothermic response. Such observations would not be anticipated if cobalt causes a lowering of the set point.

Tremorine (1,4-dipyrolidino-2-butyne) elicits profound hypothermia in mice (7). The temperature response to tremorine is dependent on the ambient temperature (8). Oxotremorine, the active metabolite of tremorine, produces marked hypothermia in mice through a cholinergic action on the CNS (9). The hypothermic response to oxotremorine in rats

Table III—Effect of Pretreatment with Various Anticholinergic Agents on the Hypothermic Response to Cobaltous Chloride in Mice

Pretreatment	Dose, mg/kg	Treatment[a]			
		Water		Cobaltous Chloride (25 mg/kg)	
		Initial Temperature	Temperature Change (Mean ± *SE*)[b]	Initial Temperature	Temperature Change (Mean ± *SE*)
Water	—	37.30°	+0.15 ± 0.10°	37.70°	−4.33 ± 0.19°
Atropine	5.0	37.26°	−0.13 ± 0.16°	37.68°	−4.01 ± 0.29°
Nicotine	0.25	37.61°	+0.15 ± 0.08°	37.67°	−4.17 ± 0.30°
Chlorpromazine	1.0	36.33°	−0.23 ± 0.10°	36.48°	−2.36 ± 0.22°[d]
Hexamethonium	10.0	37.73°	+0.07 ± 0.15°	37.61°	−4.34 ± 0.20°

[a] Pretreatments (intraperitoneal) were administered 30 min before intraperitoneal water and cobaltous chloride to groups of 12 mice. [b] Temperature changes represent the difference between body temperature recorded initially and that obtained 30 min after treatment. [c] Compared to water–water (pretreatment–treatment), $p < 0.05$. [d] Compared to water–cobaltous chloride (pretreatment–treatment), $p < 0.05$.

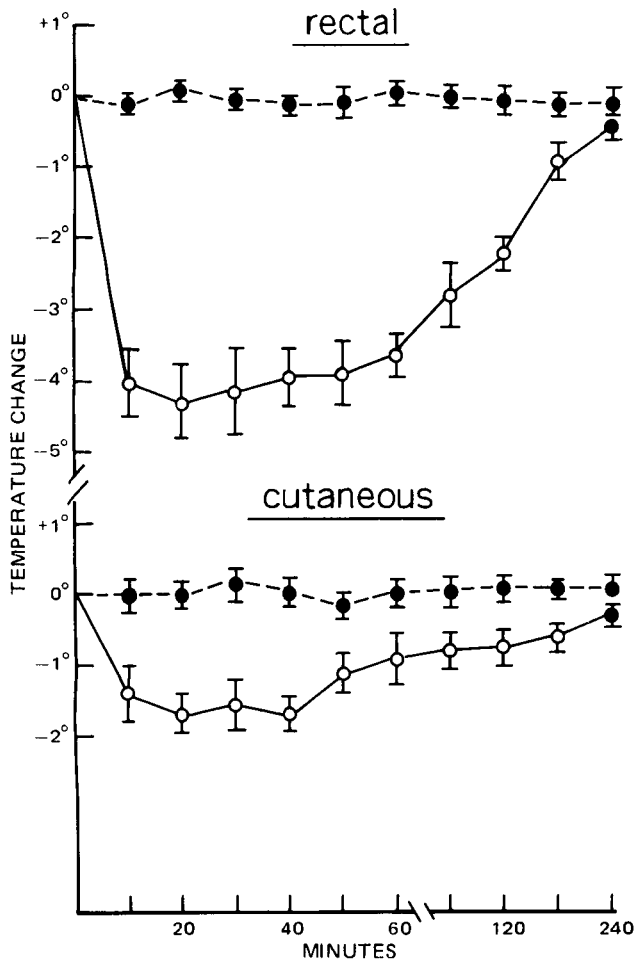

Figure 2—*Time course of the effect of distilled water (0.01 ml/g ip) (●- - -●) and cobaltous chloride (25 mg/kg ip) (●—●) on rectal and cutaneous tail temperature in mice. Water and cobaltous chloride were administered at zero time. Open symbols denote significant difference ($p < 0.05$) from water treatment at the corresponding time interval. Each point represents the average of 12 determinations. Vertical bars represent standard errors.*

was attributed to decreased heat production (10). Although not causing peripheral symptoms such as tremor, salivation, miosis, and diarrhea, cobaltous chloride exhibited similar thermoregulatory characteristics to those of oxotremorine, namely a central decrease in heat production that is dependent on the ambient temperature. On the basis of these similarities, it is possible that cobalt produces hypothermia through a central cholinergic mechanism.

Oxotremorine-induced hypothermia can be prevented in rodents by treatment with atropine (9, 11, 12). Pretreatment with atropine in the present study did not alter cobalt-induced hypothermia. Thus, it appears unlikely that cobalt produces body temperature depression through an action on central or peripheral muscarinic receptors. Likewise, the failure of nicotine and hexamethonium to prevent hypothermia precludes an influence of cobalt on central or peripheral nicotinic receptors.

Chlorpromazine was shown to partially antagonize oxotremorine-induced hypothermia in mice; this antagonism was attributed to a weak anticholinergic effect of the phenothiazine (12). Similarly, the present results showed a partial blockade of cobalt-induced hypothermia by chlorpromazine (Table III). It seems doubtful, however, that this antagonism was mediated through a central cholinolytic action, since atropine, as well as nicotine, was incapable of producing a similar blockade.

Local anesthetic and noncompetitive α-adrenergic blocking activities have been demonstrated for chlorpromazine (13). It is possible that one or both of these actions may be involved in the prevention of cobalt-induced hypothermia in mice.

REFERENCES

(1) P. Lomax and G. V. Knox, in "The Pharmacology of Thermoregulation," E. Schönbaum and P. Lomax, Eds., Karger, New York, N.Y., 1973, p. 147.

(2) D. H. Burke, *Toxicol. Appl. Pharmacol.*, in press.

(3) P. Spencer, *Life Sci.*, **5**, 1015 (1966).

(4) W. E. Kirkpatrick, *Proc. West. Pharmacol. Soc.*, **10**, 51 (1967).

(5) T. Haley and W. McCormick, *Br. J. Pharmacol.*, **12**, 12 (1957).

(6) W. Janaskovic and J. Davidovic, *Vojnosanit. Pregl.*, **14**, 530 (1957).

(7) G. M. Everett, L. E. Blockus, and I. M. Shepperd, *Science*, **124**, 79 (1956).

(8) L. E. Blockus and G. M. Everett, *Fed. Proc.*, **16**, 1209 (1957).

(9) P. S. J. Spencer, *Br. J. Pharmacol.*, **25**, 442 (1965).

(10) P. Lomax and D. J. Jenden, *Int. J. Neuropharmacol.*, **5**, 353 (1966).

(11) W. E. Kirkpatrick and P. Lomax, *Life Sci.*, **6**, 2273 (1967).

(12) D. H. Burke and D. E. Mann, Jr., *J. Pharm. Sci.*, **59**, 1814 (1970).

(13) P. Lomax and W. E. Kirkpatrick, "The Phenothiazines and Structurally Related Drugs," Raven, New York, N.Y., 1974, pp. 719–729.

ACKNOWLEDGMENTS

Presented at the Pharmacology and Toxicology Section, APhA Academy of Pharmaceutical Sciences, Phoenix meeting, November 1977.

Supported in part by a grant from the Marquette University Committee on Research.

The author is indebted to Julie Baron and Bobbi Stewart for assistance and to J. C. Brooks and G. P. O'Hara for helpful suggestions.