change $\Delta H_{T,s}$ [mean value ($\pm SD$) of 6.75 ($\pm$0.48) kcal/mole], although the more hydroxy groups added, the higher the aqueous solubility. The C_s value increased 1.25–11.29-fold.

The data in Table III indicated that the addition of an hydroxy group at position 21 remarkably affected the solution solubility (C_s). With the addition of a 21-OH group, the C_s increased almost fourfold, from 353.3 ($\pm$16.2) (I) to 1402.1 ($\pm$75.4) (II) μg/ml. The significance of the 21-OH group in progesterone derivative solubility enhancement can be demonstrated by removing the 21-OH group by acetylation. Desoxycorticosterone acetate formation reduced C_s substantially to 249.9 ($\pm$2.1) μg/ml. The reduction of C_s was accompanied by an increase in $\Delta H_{T,s}$, the solvation energy, from 7.17 to 10.99 kcal/mole. For desoxycorticosterone acetate, C_s and $\Delta H_{T,s}$ were very close to the value for progesterone (353.3 ± 16.2 μg/ml and 10.22 kcal/mole, respectively). A similar result was achieved when the 21-OH group of hydrocortisone (VII) was acetylated to form hydrocortisone acetate.

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Cobaltous Chloride-Induced Hypothermia II: Pretreatment with Sympathoplegics, Antihistamines, and Narcotic Antagonists

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Received August 25, 1978, from the *Divisions of Pharmacology and Physiology, School of Dentistry, Marquette University, Milwaukee, WI 53233.* Accepted for publication November 13, 1978.

Abstract □ Body temperature depression was noted in rats, mice, and hamsters following intraperitoneal cobaltous chloride administration (25 mg/kg). Intracerebral cobalt injection elicited hypothermia in rats and mice but not in hamsters. Body temperature depression appeared to be centrally mediated in rats and mice and peripherally mediated in hamsters. The effect of intraperitoneal and intracerebral pretreatment with phentolamine, diphenhydramine, propranolol, cimetidine, and naloxone on the mouse rectal temperature response to cobalt (25 mg/kg ip) was noted. Systemic phentolamine injection (intraperitoneal) did not alter the cobalt response, whereas intracerebral administration partially antagonized cobalt-induced hypothermia, indicating that antagonism was mediated centrally. Pretreatment with propranolol and cimetidine failed to modify the temperature response. Intracerebral diphenhydramine did not influence cobalt hypothermia. However, this agent reduced the cobalt response when given intraperitoneally, presumably through a peripheral inhibitory mechanism. The intracerebral injection of naloxone 30 min prior to cobalt slightly enhanced hypothermia, apparently through a central action. Intracerebral 6-hydroxydopamine injection depleted brain norepinephrine and dopamine but exhibited no apparent influence on cobalt-induced hypothermia.

Keyphrases □ Hypothermia—cobaltous chloride induced, effect of phentolamine, diphenhydramine, propranolol, cimetidine, naloxone, rats, mice, hamsters, species specificity, central *versus* peripheral effects □ Cobaltous chloride—hypothermia, rats, mice, hamsters, effect of phentolamine, diphenhydramine, propranolol, cimetidine, naloxone, species specificity □ Sympathoplegics—effect on cobaltous chloride-induced hypothermia □ Antihistamines—effect on cobaltous chloride-induced hypothermia □ Narcotic antagonists—effect on cobaltous chloride-induced hypothermia

Many agents interfere with thermoregulatory control as a result of their influence on the central nervous system (CNS). Intracerebral dopamine and norepinephrine injections into conscious mice caused hypothermia (1). Similarly, histamine and oxotremorine injection into the lateral ventricles produced a dose-related fall in body temperature (2–4). Hypothermia, due solely to a decrease in heat production, was reported following a single central morphine injection in rats (5, 6).

Recent studies in this laboratory showed that cobaltous

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Table I—Body Temperature Changes 30 min after Itracerebral Cobaltous Chloride Injection in Various Species

Species	Water[a] Initial Temperature	Water[a] Temperature Change (Mean ± SE)[b]	Cobaltous Chloride[a] Initial Temperature	Cobaltous Chloride[a] Temperature Change (Mean ± SE)[b]
Mice	37.19°	−0.12 ± 0.04°	37.24°	−4.08 ± 0.19°[c]
Rats	37.67°	+0.03 ± 0.09°	37.69°	−3.56 ± 0.18°[c]
Hamsters	37.24°	−0.26 ± 0.13°	37.46°	−0.18 ± 0.16°

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

chloride causes pronounced hypothermia in mice, apparently through a centrally mediated decrease in heat production (7). Although pretreatment with atropine, hexamethonium, and nicotine failed to modify the cobalt response, a partial antagonism was noted after pretreatment with chlorpromazine.

This paper describes cobalt-induced hypothermia and modification of this response by pretreatment with histaminergic, noradrenergic, or "opioid" receptor blocking agents.

EXPERIMENTAL

Animals—The experiments were performed on male Swiss albino mice, 18–25 g, on male Wistar rats, 140–160 g, and on male Syrian hamsters, 50–70 g. The animals were housed in groups of six to 20 with *ad libitum* access to laboratory food[1] and water for at least 3 days prior to use. For 24 hr before and including the time of the experiment, the animals were kept in a room free from drafts at a constant environmental temperature (23 ± 1°).

Drugs—Fresh drug and chemical solutions were prepared with distilled water in concentrations (calculated as the salt) 0.01 ml/g of body weight. Intracerebral injections were administered in a fixed volume of 0.01 ml/animal. Cobaltous chloride[2], phentolamine mesylate[3], propranolol hydrochloride[4], diphenhydramine hydrochloride[5], cimetidine hydrochloride[6], naloxone hydrochloride[7], and 6-hydroxydopamine[8] were used.

Body Temperature Recording—A thermistor thermometer[9] was used for obtaining body temperature. Rectal temperatures were recorded with a thermistor probe inserted ~2.5 cm (5 cm for rats) and held in position until constant readings were attained.

At the start of daily testing, the animals were placed singly in circular wire-mesh cages and individual weights were recorded with a triple-beam balance[10]. Immediately following weight determination, initial temperatures were recorded and treatments were administered. Temperatures were recorded again at various intervals. Unless otherwise stated, treatments were intraperitoneal. Control animals received distilled water (0.01 ml/g). To study the influence of various drugs on cobalt-induced hypothermia, pretreatment injections (water, phentolamine, propranolol, diphenhydramine, cimetidine, naloxone, and 6-hydroxydopamine) were given at specified intervals prior to recording initial temperatures.

Intracerebral Injections—Intracerebral injections were carried out according to a literature method (8). Agents were injected 2 mm lateral to the midline on a line joining the anterior bases of the ears. A 27-gauge needle attached to a microliter syringe[11] was inserted perpendicularly through the skull to a depth of 3 mm. Similar prior injection of several animals with methylene blue (0.5%) resulted in dye localization in the third and fourth ventricles.

[1] Wayne Lab-Blox.
[2] J. T. Baker Chemical Co.
[3] Ciba Pharmaceutical Co.
[4] Ayerst Laboratories.
[5] Parke-Davis & Co.
[6] SK&F Lab Co.
[7] Endo Laboratories.
[8] Sigma Chemical Co.
[9] Model 46 Tele-Thermometer, Yellow Springs Instrument Co., Yellow Springs, Ohio.
[10] Ohaus.
[11] Hamilton.

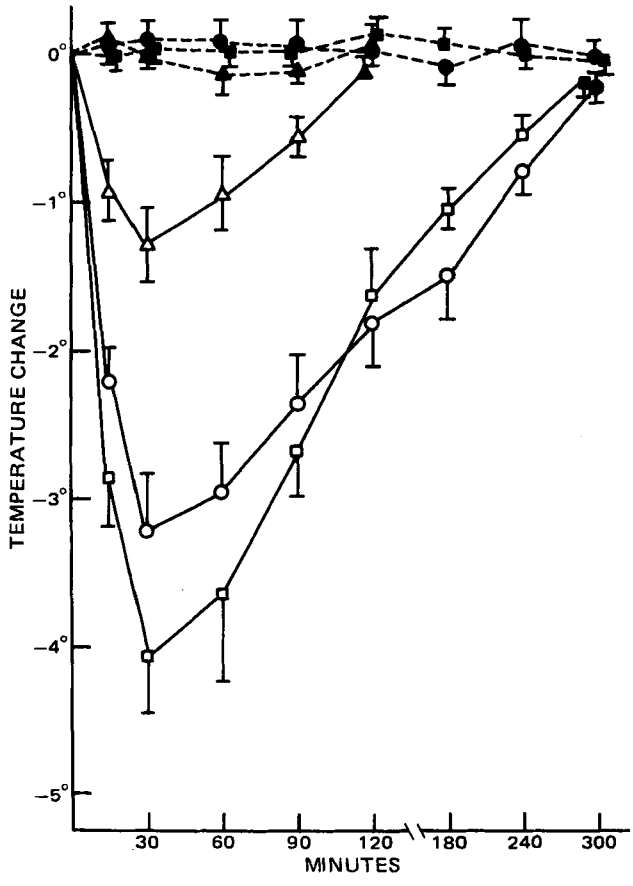

Figure 1—*Time course of the effect of 0.01 ml/g ip distilled water (- - -) and cobaltous chloride (25 mg/kg ip) (—) on rectal temperature in hamsters (▲), rats (●), and mice (■). Water or cobaltous chloride were administered at zero time. Open symbols denote significant difference ($p < 0.05$) from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

Catecholamine Assay—Dopamine and norepinephrine were extracted from the brain and quantitated as the trihydroxyindoles (9). Amines were isolated from deproteinized brain homogenates by chromatography on a cation-exchange resin[12]. The individual amines were quantitated fluorometrically[13] with standard curves relating relative fluorescence to concentration.

Statistics—Results are expressed as mean ± standard error. Significance was calculated using the Student *t* test. Differences were considered significant at the probability level of 5% or less.

RESULTS

Core temperature changes were recorded at various intervals for 5 hr following cobaltous chloride administration (25 mg/kg ip) to unrestrained mice, rats, and hamsters (Fig. 1). Maximum body temperature depression (~1.2°) was evident 30 min after cobalt injection in hamsters. Hypothermia duration in this species was ~90 min. Cobaltous chloride caused more body temperature depression in rats (3.2°) and mice (4.1°). In each case, maximum hypothermia occurred within 30 min. A 4-hr hypothermia duration was noted in rats and mice.

Body temperature changes were noted 30 min after intracerebral cobaltous chloride injection (25 μg) in mice, rats, and hamsters (Table I). Mice and rats exhibited respective drops of 4.08 and 3.56°. Visible shivering and increased muscle tone were absent throughout the body temperature response. In both species, hypothermia was accompanied by depression of locomotor activity. Cobaltous chloride failed to elicit a significant body temperature depression when injected intracerebrally in hamsters.

[12] Dowex 50-X8.
[13] Aminco-Bowman SPF spectrophotofluorometer.

Table II—Effect of Intraperitoneal (ip) and Intracerebral (ic) Pretreatment with Various Agents on the Hypothermic Response to Cobaltous Chloride in Mice

Pretreatment[a]	Route	Dose	Water		Cobaltous Chloride (25 mg/kg)	
			Initial Temperature	Temperature Change (Mean ± SE)[b]	Initial Temperature	Temperature Change (Mean ± SE)[b]
Water	ip	—	37.36°	+0.01 ± 0.07°	37.45°	−4.28 ± 0.22°[c]
Water	ic	—	37.11°	−0.10 ± 0.07°	37.08°	−3.93 ± 0.11°[d]
Phentolamine mesylate	ip	10 mg/kg	37.20°	−0.12 ± 0.10°	37.21°	−4.14 ± 0.25°
Phentolamine mesylate	ic	10 μg	36.72°	−0.24 ± 0.07°	36.85°	−3.05 ± 0.15°[e]
Propranolol hydrochloride	ip	10 mg/kg	37.13°	+0.10 ± 0.08°	37.54°	−4.68 ± 0.23°
Propranolol hydrochloride	ic	10 μg	36.59°	−0.21 ± 0.15°	36.78°	−4.06 ± 0.14°
Diphenhydramine hydrochloride	ip	5 mg/kg	37.24°	+0.09 ± 0.08°	37.38°	−3.48 ± 0.14°[f]
Diphenhydramine hydrochloride	ic	5 μg	37.10°	+0.03 ± 0.08°	37.07°	−3.85 ± 0.14°
Cimetidine hydrochloride	ip	30 mg/kg	37.36°	−0.05 ± 0.07°	37.46°	−4.13 ± 0.13°
Cimetidine hydrochloride	ic	30 μg	37.42°	−0.17 ± 0.09°	37.12°	−4.03 ± 0.16°
Naloxone hydrochloride	ip	1 mg/kg	37.60°	−0.06 ± 0.05°	37.96°	−4.18 ± 0.12°
Naloxone hydrochloride	ic	1 μg	37.44°	−0.02 ± 0.09°	37.30°	−4.45 ± 0.11°[e]

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

Table III—Effect of Intracerebral Pretreatment with 6-Hydroxydopamine on the Hypothermic Response to Cobaltous Chloride in Mice

Pretreatment[a]	Pretreatment–Treatment Interval, days	Water (0.01 ml/g ip)		Cobaltous Chloride (25 mg/kg ip)	
		Initial Temperature	Temperature Change (Mean ± SE)[b]	Initial Temperature	Temperature Change (Mean ± SE)[b]
Vehicle[c]	14	38.63°	−0.31 ± 0.11°	38.56°	−5.31 ± 0.25°[d]
6-Hydroxydopamine	14	38.84°	−0.36 ± 0.10°	38.31°	−5.42 ± 0.30°
Vehicle	42	38.62°	−0.39 ± 0.11°	38.79°	−5.41 ± 0.30°[e]
6-Hydroxydopamine	42	38.78°	−0.30 ± 0.07°	38.81°	−5.34 ± 0.32°

[a] Pretreatments (intracerebral) were administered 14 and 42 days before intraperitoneal water and cobaltous chloride to groups of 18 mice. [b] Temperature changes represent the difference between body temperature recorded initially and that obtained 30 min after treatment. [c] Aqueous ascorbic acid solution (0.5%) with pH adjusted to 5.5. [d] Compared to vehicle–water (pretreatment–treatment) at the 14-day pretreatment–treatment interval, $p < 0.05$. [e] Compared to vehicle–water (pretreatment–treatment) at the 42-day pretreatment–treatment interval, $p < 0.05$.

The effect of intraperitoneal and intracerebral pretreatment with phentolamine mesylate, propranolol hydrochloride, diphenhydramine hydrochloride, cimetidine hydrochloride, and naloxone hydrochloride on the body temperature response to cobaltous chloride (25 mg/kg) in mice is shown in Table II. Pretreatment doses were incapable of causing significant body temperature alteration. Intracerebral phentolamine administration partially attenuated the hypothermic response to cobalt. Phentolamine produced no significant effect upon cobalt hypothermia when given intraperitoneally. Propranolol did not modify cobalt-induced hypothermia by either the intraperitoneal or intracerebral route. Systemic diphenhydramine administration partially inhibited cobalt hypothermia. No antagonism occurred following intracerebral administration. Cobaltous chloride-induced body temperature depression was not significantly altered by intraperitoneal naloxone, but a hypothermia potentiation was observed after intracerebral naloxone.

Table III describes the effect of intracerebral 6-hydroxydopamine pretreatment (100 μg) on the hypothermic response to cobaltous chloride (25 mg/kg ip) in mice. Pretreatment 14 and 42 days prior to cobalt treatment did not affect the body temperature response.

At 14 days following intracerebral 6-hydroxydopamine injection, mouse brain norepinephrine and dopamine concentrations were reduced by 59.09 and 71.43%, respectively (Table IV). A similar reduction in catecholamine concentrations was noted 42 days after the central administration of 6-hydroxydopamine.

DISCUSSION

The body temperature response to cobaltous chloride was species dependent. Hypothermia intensity and duration were greater in rats and mice than in hamsters.

Intracerebral cobalt injection did not produce hypothermia in hamsters but did produce body temperature depression in mice and rats, apparently through a central mechanism. The small drop in hamster body temperature produced by intraperitoneal injection was probably due to peripheral activity.

Since catecholamine, histamine, and opioid receptor stimulation within the CNS is associated with hypothermia in mice (1, 10–12), it seemed logical that cobalt might accomplish body temperature depression through its influence on one or more of these receptive sites. Intracerebral, but not systemic, phentolamine partially antagonized cobalt-induced hypothermia. It is possible, therefore, that cobalt hypothermia is mediated, in part, either through a direct influence on α-adrenergic receptors or through norepinephrine release within the CNS.

The absence of an intracerebral or intraperitoneal propranolol effect on cobalt hypothermia indicates that cobalt does not act through central or peripheral β-receptors.

The H_1 and H_2 receptor blocking agents diphenhydramine and cimetidine did not modify cobalt hypothermia when injected intracerebrally and intraventricularly, respectively. Cobalt hypothermia apparently is not mediated through activity upon central H_1 or H_2 receptors.

On the other hand, intraperitoneal diphenhydramine partially antagonized the temperature response to cobalt. Cobalt-induced hypothermia may be mediated, in part, peripherally either through histamine release or through a direct influence on H_1 receptors.

Intracerebral naloxone slightly enhanced cobalt-induced hypothermia, apparently through a central action.

The catecholamine-depleting agent 6-hydroxydopamine markedly reduced brain norepinephrine and dopamine levels (~59 and 72%, respectively) at 2 and 6 weeks following pretreatment (Table IV). However, 6-hydroxydopamine had no effect on cobalt hypothermia. It is possible, therefore, that cobalt produces hypothermia through mechanisms other than norepinephrine or dopamine release. Over a period of time, however, receptors for these amines may have become "supersensitive." Thus, less released norepinephrine or dopamine could have caused a more intense response.

Little information is available concerning brain dopamine depletion in mice following central 6-hydroxydopamine administration. Tabakoff and Ritzmann (13) reported that intracerebrally administered 6-hydroxydopamine (50 μg) depleted norepinephrine but had little effect on dopamine and serotonin levels. The present results show that intracerebral 6-hydroxydopamine (100 μg) depleted mouse brain dopamine.

Previous experiments in this laboratory showed that cobalt-induced hypothermia is partially attenuated by chlorpromazine pretreatment (7). Antagonism in the case of this phenothiazine was not mediated through a central anticholinergic action, since atropine, as well as nicotine, failed to achieve a similar blockade. In addition to anticholinergic activity,

Table IV—Intracerebral 6-Hydroxydopamine Effect on Norepinephrine and Dopamine Concentration in Mice Brains

Days after Injection[a]	Norepinephrine			Dopamine		
	Vehicle[b], μg/g ± SE	6-Hydroxydopamine, μg/g ± SE	Change, %	Vehicle, μg/g ± SE	6-Hydroxydopamine, μg/g ± SE	Change, %
14	0.308 ± 0.02	0.126 ± 0.03[c]	−59.09	0.287 ± 0.03	0.082 ± 0.03[c]	−71.43
42	0.336 ± 0.01	0.135 ± 0.03[c]	−59.82	0.298 ± 0.02	0.082 ± 0.02[c]	−72.48

[a] The mice brains (groups of six) were removed and assayed for catecholamine content 14 and 42 days following the intracerebral water or 6-hydroxydopamine injection (100 μg). [b] Aqueous ascorbic acid solution (0.5%) with pH adjusted to 5.5. [c] Compared to the appropriate vehicle, $p < 0.05$.

chlorpromazine possesses adrenolytic and antihistaminic qualities (14). Chlorpromazine may partially reverse the cobalt response through a central α-receptor blockade and/or peripheral antihistaminic activity.

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Antifertility and Antiproteolytic Activity of Activated *N*-Carbobenzoxy Amino Acid Esters

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Abstract □ *N*-Carbobenzoxy L-phenylalanine, glycine, L-leucine, and L-proline derivatives, their vinyl esters, and their 1,2-dibromoethyl esters were tested for antifertility activity in mice. Intraperitoneal administration reduced the pregnancy percentage and the number of fetuses per litter. Intravaginal administration reduced the pregnancy percentage significantly, with *N*-carbobenzoxyglycine vinyl ester, *N*-carbobenzoxyglycine-1,2-dibromoethyl ester, *N*-carbobenzoxy-L-leucine-1,2-dibromoethyl ester, and *N*-carbobenzoxy-L-proline-1,2-dibromoethyl ester producing 100% inhibition at 10 mg/kg/day. Sperm enzyme hydrolysis of the nonspecific substrate azocasein was inhibited significantly by certain *N*-carbobenzoxy amino acid esters *in vitro*. Specific substrate *N*-benzoyl-L-arginine ethyl ester hydrolysis was also inhibited. Compounds that inhibited *N*-benzoyl-L-arginine ethyl ester hydrolysis also demonstrated *in vivo* intravaginal antifertility activity.

Keyphrases □ Carbobenzoxy amino acid esters—contraceptive activity, antiproteolytic activity, mice □ Contraceptives, potential—activated *N*-carbobenzoxy amino acid esters, mice □ Enzyme activity—effect of activated *N*-carbobenzoxy amino acid esters on sperm proteolysis, mice □ Sperm—enzymes, effect of *N*-carbobenzoxy amino acid esters, mice

Recently, numerous activated *N*-protected amino acid esters were synthesized as possible latent proteolytic inhibitors (1, 2). These agents resemble known trypsin and chymotrypsin inhibitors. Certain members of the series effectively inhibited *in vivo* cathepsin activity and *in vitro* chymotrypsin activity (3). Since proteolytic enzyme inhibitors block sperm acrosin activity, capacitation, and fertility, the effects of this series on reproduction were investigated.

EXPERIMENTAL

Chemistry—*N*-Carbobenzoxy-L-phenylalanine (I), *N*-carbobenzoxyglycine (II), *N*-carbobenzoxy-L-leucine (III), and *N*-carbobenzoxy-L-proline (IV) were prepared by the standard procedure (4) (Table I). The *N*-carbobenzoxy amino acids were converted to the corresponding vinyl esters V, VI, VII, and VIII by refluxing with vinyl acetate in the presence of palladium chloride and were purified by column chromatography. The vinyl esters were treated with bromine in chloroform to give the 1,2-dibromo esters IX, X, XI, and XII, which were purified by fractional crystallization or column chromatography (1, 2). *N*-α-Tosyl-L-lysylchloromethyl ketone (XIII), tosyl-L-phenylalanylchloromethyl ketone (XIV), carbobenzoxy-L-phenylalanine chloromethyl ketone (XV), 17-ethinyl estradiol (XVI), and diethylstilbestrol (XVII) were purchased[1].

Antifertility Screens—For 28 days, virgin CF_1 female mice (~28 g), which had been isolated for 4 weeks, were administered test compounds suspended in 1% carboxymethylcellulose at 10 mg/kg/day (0.2 ml) ip. On Day 10, female mice were exposed to male mice (two females per male) for the remainder of the experiment. Male mice were rotated once a week to assure fertility. On gestation Days 17–21, the females were sacrificed;

[1] Sigma Chemical Co. or Cyclo Chemicals.

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