in the experiment by Levy and Gucinski (9) the fish exposed to secobarbital apparently did fit the model, which led these investigators to conclude that secobarbital was absorbed in both the unionized and ionized forms. For the most part, that experiment was similar to the controls in this experiment.

In summary, there appears to be a period of time after exposure of fish to the chelating agent in which the enhanced permeability of the membrane to the barbiturate is absent. However, once the effect is obtained, it is present 24 hr after exposure. The chelating agent appears to affect the transport of the ionized drug species, but the increase in the transport of the ionized form is not enough to compensate for the reduction of the unionized concentration in the pH study. There also appears to be a direct relationship between the change in permeability and the concentration of chelating agent to which the fish are exposed. Further studies are underway concerning the effects of edetate disodium on the goldfish membrane.

APPENDIX

The model proposed by Levy and Gucinski (9) concerning the absorption of both the unionized and ionized drug forms in the goldfish is:

$$R = K_i C_i + K_u C_u \qquad \text{(Eq. A1)}$$

where R is the absorption rate, K_i and K_u are the first-order rate constants, and C_i and C_u are the concentrations of the ionized and unionized species, respectively. The amount of drug in the fish is equal to the product of the absorption rate and the time the fish is exposed to the bathing solution:

$$\text{amount} = Rt \qquad \text{(Eq. A2)}$$

Substituting Eq. A1 for R in Eq. A2 yields:

$$\text{amount} = (K_i C_i + K_u C_u)t \qquad \text{(Eq. A3)}$$

which is equivalent to:

$$1/t = \frac{K_i C_i + K_u C_u}{\text{amount}} \qquad \text{(Eq. A4)}$$

Since the sum of C_i and C_u represents the total drug concentration, which was constant for these experiments, then:

$$1/t = \frac{C}{\text{amount}}[K_i(1 - f) + K_u f] \qquad \text{(Eq. A5)}$$

where C represents the total concentration of drug and f and $1 - f$ represent the fraction of the drug present in the unionized and ionized forms, respectively. Therefore:

$$1/t = \frac{C}{\text{amount}}(K_i - K_i f + K_u f) \qquad \text{(Eq. A6)}$$

and:

$$1/t = \frac{CK_i}{\text{amount}} + \frac{C(K_u - K_i)f}{\text{amount}} \qquad \text{(Eq. A7)}$$

If it is assumed that the amount of drug necessary to produce death is constant regardless of the pH employed:

$$1/t = K_b K_i + K_b(K_u - K_i)f \qquad \text{(Eq. A8)}$$

where K_b is a constant and equals C/amount. Therefore, a plot of the reciprocal of the response time ($1/t$) as a function of the fraction of drug unionized should be linear with a slope equal to $K_b(K_u - K_i)$ and an intercept equal to $K_b K_i$.

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Stability of Apomorphine in Solutions Containing Ascorbic Acid and Bisulfite and Effects of Antioxidants on Apomorphine-Induced Cage Climbing and Hypothermia in Mice

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Received December 10, 1979, from the **Department of Pharmacology and* ‡*Drug Dynamics Institute, College of Pharmacy, University of Texas at Austin, Austin, TX 78712.* Accepted for publication February 29, 1980.

Abstract □ Ascorbic acid (100 mg/ml) and sodium bisulfite (0.5 and 20 mg/ml) prevented more than 10% oxidation of apomorphine hydrochloride in water maintained at room temperature over 1–3 days. Refrigeration at 5° prevented oxidation of apomorphine hydrochloride in aqueous solutions for 1 week. Neither ascorbic acid nor sodium bisulfite affected murine stereotyped cage climbing or hypothermia induced by apomorphine.

Keyphrases □ Apomorphine—stability in solutions containing ascorbic acid and sodium bisulfite, effects of antioxidants on apomorphine-induced cage climbing and hypothermia in mice □ Antioxidants—ascorbic acid and sodium bisulfite, stability of apomorphine in solutions containing antioxidants, effects of antioxidants on apomorphine-induced cage climbing and hypothermia in mice □ Stability—apomorphine in solutions containing ascorbic acid and sodium bisulfite

Current interest in apomorphine (I) stems from its activity as a dopaminergic agonist and its consequent antiparkinsonian activity (1–3). Its clinical unity has been demonstrated when used alone and in combination with other agents such as levodopa (4). In addition to parkinsonism, recent studies indicated potential new uses for I

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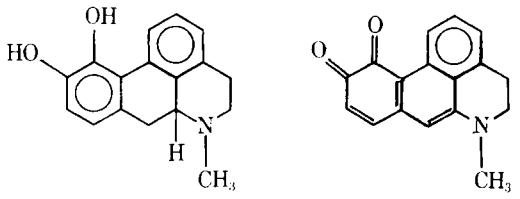

I

II

in treating Huntington's chorea (5), tardive dyskinesia (6, 7), Gilles de la Tourette's syndrome (8), and schizophrenia (9, 10).

BACKGROUND

Recent efforts have focused on extending the pharmacokinetic studies of I in mice. The disposition of I following parenteral administration to mice was reported (11–14). Study of the plasma levels of I in mice following intraperitoneal, intravenous, and oral administrations (15) and preliminary investigations (15, 16) suggested a correlation between circulating levels of I and stereotyped cage climbing (16). Continued work is aimed at extensive comparisons between circulating levels of I and the stereotyped cage climbing and hypothermia induced by I in mice. These studies are being conducted to develop pharmacological models that might be useful in evaluating the efficacy of apomorphine prodrugs, some of which already have been synthesized (17) and evaluated pharmacologically in rats (18).

A problem complicating work with apomorphine is its tendency to undergo air oxidation. Aqueous solutions of I·HCl rapidly become green to blue green due to the accumulation of oxidation products (19). The oxidation process in neutral and acidic media is relatively uncomplicated, leading principally to the orthoquinone, II (20, 21). However, at alkaline pH values, the degradation is considerably more complex and yields four or more products (22, 23).

Burkman (24, 25) studied the oxidation kinetics of I from pH 5.2 to 6.8. The half-lives of I in buffered solutions were 11–166 hr with the more acidic solutions being more stable. An unbuffered 1.2% solution of I·HCl (pH 5.5) was considerably more stable than its buffered counterpart. Various investigators recommended the use of citrate (26), bisulfite (27), ascorbic acid (28), thiourea (29), and edetic acid (26) to stabilize aqueous solutions of apomorphine.

The purpose of this investigation was to reevaluate the use of bisulfite and ascorbate in stabilizing I solutions maintained at room temperature and under refrigerated conditions. The use of a specific high-performance liquid chromatographic (HPLC) assay, developed recently in these laboratories (30), was expected to facilitate this work. Furthermore, studies of the effect of ascorbate and bisulfite on apomorphine-induced stereotyped cage climbing and hypothermia in mice were conducted to determine if the antioxidants interfered in these test systems.

EXPERIMENTAL

Reagents—(*R*)-(−)-Apomorphine hydrochloride hemihydrate[1] [98% pure as determined by UV spectrophotometry (31)], ascorbic acid[2], and sodium bisulfite[2] were used as purchased. Apomorphine orthoquinone (6-methyl-5,6-dihydro-4*H*-dibenzo[*de,g*]quinone-10,11-dione) (II) was prepared according to a literature method (20). The methanol and acetonitrile used in the HPLC studies were distilled in glass[3]. All other solvents and reagents were analytical reagent grade. Water was double distilled in glass.

HPLC Analyses—The apomorphine content of solutions was analyzed using a previously described HPLC method (30). For this application, a UV detector[4] was used at 273 nm with a setting of 1.28 aufs. Under the analytical conditions, authentic II, ascorbic acid, and sodium bisulfite showed no interference in the region of the developed apomorphine peak.

Stability Studies—Samples of I·HCl were prepared at a concentration of 0.2 mg/ml in water, in a 1-mg/ml aqueous ascorbic acid solution, in a 100-mg/ml aqueous ascorbic acid solution, in a 0.52-mg/ml aqueous sodium bisulfite solution, and in a 20-mg/ml aqueous sodium bisulfite solution. Blank and sample solutions were prepared in clear 10-ml glass-stoppered containers and maintained at room temperature (22–25°) under typical laboratory conditions of light exposure. Samples were analyzed in triplicate at time zero and 1, 3, 7, and 8 days following preparation by direct injection of 100-μl portions into the HPLC column. Quantitation was performed by comparison of peak heights to a calibration curve obtained (r = 0.999) from chromatographic development of five freshly prepared solutions of apomorphine (in 1-mg/ml ascorbic acid solution) containing 0.02–0.2 mg of I·HCl/ml.

Refrigerator Stability—This experiment was performed as was the previous study, except that three apomorphine solutions (in water alone, in a 100-mg/ml ascorbic acid solution, and in a 20-mg/ml sodium bisulfite solution) were kept in a laboratory refrigerator (5°) until sampling.

Pharmacological Studies—Experimentally naive CD-1 male mice[5] weighed 20–36 g at the time of testing. Access to food and water was provided *ad libitum*, and the animals were maintained in a 12-hr light–dark cycle (lights on from 6 am to 6 pm); all testing was done between 8 am and 5 pm.

The cage climbing and hypothermia experiments were designed similarly. Each experiment consisted of one between-subject factor, the solution (4.7 mg of I·HCl·1/2H_2O/kg in water, in 100-mg/ml ascorbate, or in 0.52-mg/ml sodium bisulfite), and one within-subject factor, the response (cage climb scores or body temperature changes after one of the three solutions of I).

Cage Climbing Behavior—A modification of the procedure of Protais *et al.* (32) was used. Animals were placed individually into cylindrical cages (12 cm diameter × 14 cm high with walls of vertical bars 2 mm in

Table I—Effects of Antioxidants on the Stability of Apomorphine Hydrochloride Solutions [a]

	Percent I Remaining[b]				
		Ascorbic Acid[d], mg/ml		Sodium Bisulfite[e], mg/ml	
Day	Water[c]	1	100	0.52	20
0	100 (3.3)	100 (0.6)	100 (0.3)	100.2(2.0)	100 (0.7)
1	86.4(3.4)	93.3(3.1)[f]	90.9(2.2)[f]	98.4(0.0)[f]	97.4(0.7)[f]
3	82.3(0.8)	87.3(0.9)[f]	93.6(1.7)[f]	94.3(2.2)[f]	93.0(0.8)[f]
7	76.9(0.5)	71.3(0.6)	—	87.0(1.0)[f]	85.2(0.3)[f]
8	63.6(0.9)	59.1(2.7)	92.0(1.4)[f]	81.5(1.0)[f]	80.3(0.8)[f]

[a] Results are expressed as the percent of drug on Day 0 (for 0.2-mg/ml solutions; see *Experimental*). [b] Mean ± *SD*, n = 3. [c] The initial pH was 5.43. [d] The initial solution pH values were 3.42 (1 mg/ml) and 2.34 (100 mg/ml). [e] The initial solution pH values were 5.61 (0.52 mg/ml) and 4.53 (20 mg/ml). [f] Significantly different than the water sample of that day (Neuman–Keuls tests, p = 0.05); samples were maintained at room temperature (22–25°).

Table II—Stability of Apomorphine Solutions Maintained at 5°

	Percent of I Remaining[a]		
Day	Water[b,c]	Ascorbic Acid[c]	Sodium Bisulfite[c]
0	100 ± 0.5	100 ± 1.3	100 ± 0.5
1	98.4 ± 0.5	99.5 ± 0.0	100.3 ± 0.3
3	97.6 ± 1.7	98.9 ± 0.0	97.6 ± 1.0
7	98.3 ± 1.7	97.0 ± 1.7	94.2 ± 0.8

[a] Results are expressed as the percent of drug on Day 0 (0.2 mg/ml; see *Experimental*). [b] Mean ± *SD*, n = 3. [c] No significant decomposition (analysis of variance, p = 0.05).

Table III—Effects of Antioxidants on Two *In Vivo* Responses to Apomorphine Hydrochloride

Treatment	Hypothermia[b]	Cage Climb Score[c]
I–water[a]	3.7 ± 0.5°	12.7 ± 1.4
I–ascorbic acid	4.0 ± 0.4°	11.8 ± 1.8
I–sodium bisulfite	3.2 ± 0.4°	13.8 ± 1.4

[a] A solution of 4.7 mg of apomorphine hydrochloride hemihydrate was prepared in distilled water or in aqueous solutions of ascorbate (100 mg/ml) or sodium bisulfite (20 mg/ml). [b] Body temperature changes were recorded 60 min after drug administration; analysis of variance showed no significant effects (p = 0.05). [c] Cumulative stereotypic activity for 12 consecutive 5-min periods after drug administration (maximum = 24); analysis of variance showed no significant effects (p = 0.05).

[1] McFarland Smith Ltd., Edinburgh, Scotland.
[2] Sigma Chemical Co., St. Louis, Mo.
[3] Burdick & Jackson Laboratories, Muskegon, Mich.
[4] Model 995, Tracor Instruments, Austin, Tex.
[5] Charles River Laboratories, Wilmington, Mass.

diameter and 1 cm apart) surmounted by fine wire mesh for a 20-min habituation period. Animals then received a pretreatment consisting of isotonic saline (0.1 ml/10 g of body weight ip). One hour later, the behavior of the animals was recorded on videotape as described previously (33) to establish a baseline of activity for each subject. The mice then were injected immediately with I·HCl hemihydrate (4.7 mg/kg) in water, ascorbate, or bisulfite. The behavior of each animal was recorded on videotape for 30 sec every 5 min for 1 hr and later rated "blind" using a rating scale with 0 = four paws on cage floor, 1 = two paws holding the vertical bars of the cage, and 2 = four paws holding the vertical bars of the cage.

Hypothermia—Rectal temperatures were taken immediately prior to administration of I·HCl by insertion of a rectal probe (1.5 cm) connected to a telethermometer[6]. The mice were injected with one of the three I·HCl solutions, and temperatures were taken 60 min later.

RESULTS AND DISCUSSION

As shown in Table I, both antioxidants tested were effective in delaying apomorphine decomposition. The higher levels of each antioxidant were most effective, although neither antioxidant could delay significant degradation for more than 1–3 days when solutions were stored at room temperature. Refrigeration of apomorphine samples at 5° was effective in preventing decomposition for 0–7 days, and the addition of antioxidants was unnecessary under these conditions (Table II).

Table III shows the lack of influence of the antioxidants, ascorbic acid and sodium bisulfite, on apomorphine-induced hypothermia and stereotyping of cage climbing. Neither ascorbate nor sodium bisulfite displayed any significant effects on these *in vivo* responses to I.

In summary, ascorbic acid (100 mg/ml) and sodium bisulfite (0.52 and 20 mg/ml) prevented more than 10% oxidation of apomorphine hydrochloride in water maintained at room temperature for 1–3 days. Refrigeration at 5° prevented oxidation of I·HCl in aqueous solutions for over 1 week. Neither ascorbic acid nor sodium bisulfite affected murine stereotyped cage climbing or hypothermia induced by apomorphine.

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ACKNOWLEDGMENTS

Supported by Grant NS-12259 from the National Institute of Neurological and Communicative Disorders and Stroke. D. W. Humphrey is grateful for fellowship support from Hoffmann-La Roche Inc.