Study of the Sympathomimetic Action of Cyclohexylamine, a Possible Metabolite of Cyclamate

By H. I. YAMAMURA, I. P. LEE, and R. L. DIXON

The pharmacological actions of cyclohexylamine on the autonomic nervous system were tested using the cat's blood pressure and nictitating membrane responses. Although cyclohexylamine had no effect upon the adrenergic, cholinergic, or histaminic responses as shown by epinephrine, norepinephrine, acetylcholine, or histamine, it was demonstrated to possess a pressor action, and this pressor activity could be blocked by phenoxybenzamine. The results indicated that cyclohexylamine may act by releasing the neural humoral transmitter substance norepinephrine because prior pretreatment with reserpine, which depletes the catecholamine stores, abolished the pressor effect. Upon subsequent administration of norepinephrine the pressor response returned. It was shown that there was no potentiation between cyclohexylamine and a monoamine oxidase inhibitor, but potentiation did occur between tyramine and the monoamine oxidase inhibitor.

CYCLAMATE AND saccharin are extensively used as noncaloric sweetening agents for various beverages and foods. Ingestion of cyclamate in excess of 5 Gm. per day is not an unreasonable amount considering its widespread use. Recently, Kojima and Ichibagase (1) and Leahy *et al.* (2) have indicated the possibility that a small portion of administered sodium cyclamate may be metabolized to cyclohexylamine. The possibility also exists that cyclohexylamine may be ingested either as impurity or due to spontaneous breakdown in various food products. These findings prompted this investigtion of cyclohexylamine in order to: (*a*) investigate the action of cyclohexylamine on the vascular system, (*b*) study the effect of cyclohexylamine on the action of various endogenous transmitter substances, and (*c*) consider the possible interaction between cyclohexylamine and monoamine oxidase inhibitors.

METHODS

Twenty-five cats of either sex, weighing between 2.5–3.5 Kg., were anesthetized with sodium pentobarbital (30 mg./Kg.) administered intraperitoneally (i.p.). Blood pressure was recorded from the cannulated left femoral artery using a physiograph "Four" recorder, E & M Pressure Transducer (model P-1000) and E & M Myograph (type B). The contractions of the nictitating membrane were determined after placing it under an initial tension of 5 Gm. A tracheal cannula was routinely inserted and artificial respiration was administered when necessary.

Drugs—The following drugs were used: *l*-epinephrine bitartrate (Sigma Chemical Co.); *l*-norepinephrine bitartrate (City Chemical Co.); tyramine HCl (Mann Research Laboratories); acetylcholine chloride (NBC); histamine phosphate (Burroughs Wellcome & Co.); cyclohexylamine HCl (K & K Laboratories, Inc.); reserpine (Ciba Pharmaceutical Co.); pargyline HCl (Abbott Laboratories); phenoxybenzamine HCl (Smith Kline and French Laboratories); and propranolol (Ayerst Laboratories). The concentrations of drugs used in all experiments were expressed on the basis of their salts, except for epinephrine and norepinephrine, which were expressed on the basis of their base forms.

RESULTS

General Action of Cyclohexylamine—Figure 1 shows a record taken from an experiment performed using an anesthetized cat. It can be seen that injections of 10 mcg./Kg. each of epinephrine and norepinephrine, 3 mcg./Kg. of acetylcholine, and 5 mcg./Kg. of histamine resulted in the characteristic responses of each drug. Cyclohexylamine (5 mg./Kg.) was then administered intravenously and a pressor response was noted. Subsequent administration of epinephrine, norepinephrine, acetylcholine, and histamine still produced their characteristic responses indicating that cyclohexylamine was not an adrenergic, cholinergic, or a histaminic blocker, but that it was capable of exerting a pressor action.

Reversal of Cyclohexylamine Response by Phenoxybenzamine—To determine if cyclohexylamine was a sympathomimetic amine, phenoxy-

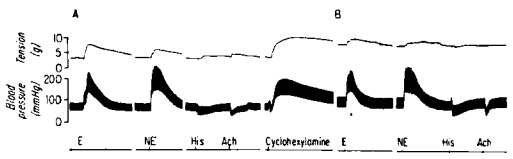

Fig. 1—The effects of epinephrine (E) (10 mcg./Kg.), norepinephrine (NE) (10 mcg./Kg.), histamine (His) (5 mcg./Kg.), and acetylcholine (ACh) (3 mcg./Kg.) before and after the administration of cyclohexylamine (5 mg./Kg.) on the cat's blood pressure and nictitating membrane.

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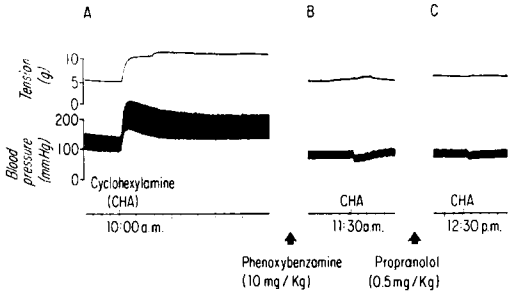

Fig. 2—The effect of cyclohexylamine (CHA) (5 mg./Kg.) on the cat's blood pressure and nictitating membrane following phenoxybenzamine (10 mg./Kg.) and propranolol (0.5 mg./Kg.).

benzamine (10 mg./Kg.), an α-adrenergic blocking agent, was administered intravenously. These results are presented in Fig. 2. After the adrenergic blockade was established, which required approximately 30 min., a second dose of cyclohexylamine (5 mg./Kg.) was given. The usual pressor response to cyclohexylamine was no longer noticed; rather a depressor response was now observed. Propranolol (0.5 mg./Kg.), a β-blocking agent, was then given to determine whether the depressor response could be blocked. Partial blockage of the depressor response was noted as seen in Fig. 2.

Effect of Cyclohexylamine After Pretreatment with Reserpine—Figure 3 demonstrates that an injection of 500 mcg./Kg. of tyramine, an indirect acting amine, caused a rise in blood pressure and produced a contraction of the nictitating membrane in a normal anesthetized cat. An injection of 5 mg./Kg. of cyclohexylamine also caused a large rise in blood pressure and a large and prolonged contraction of the nictitating membrane. In a subsequent study (Fig. 4), the successive injections of tyramine and cyclohexylamine were administered to a cat pretreated approximately 24 hr. with reserpine (1 mg./Kg. i.p.) in order to deplete the catecholamine stores. The effects of tyramine and cyclohexylamine, both on the blood pressure and nictitating membrane response, were diminished by the reserpine treatment. Norepinephrine (0.6 mg./Kg.) was then administered slowly into the femoral vein. This injection of norepinephrine caused a large rise in blood pressure and contraction of the nictitating membrane (Fig. 4). After the injection of norepinephrine, the blood pressure and contraction of the nictitating membrane returned to their initial level. An injection of tyramine (500 mcg./Kg.) followed by cyclohexylamine (5 mg./Kg.) was made again as shown in Fig. 4. The increase in the blood pressure was equal to that of control values and there was a noticeable contraction of the nictitating membrane which was absent after pretreatment with reserpine. The events shown in Fig. 4 were observed in each of three experiments. After the pressor action of tyramine and cyclohexylamine had been restored by administration of norepinephrine, the repeated injections of either tyramine or cyclohexylamine had a diminished effect. However, another injection of norepinephrine once again restored the pressor action.

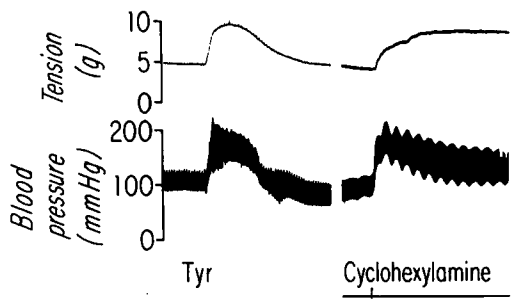

Fig. 3—The cat's pressor and nictitating membrane responses to tyramine (500 mcg./Kg.) and cyclohexylamine (5 mg./Kg.) in a normal anesthetized cat.

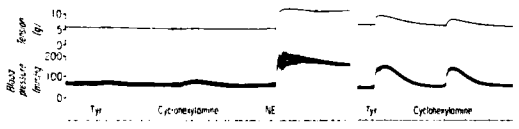

Fig. 4—The effect of slow administration of norepinephrine (0.6 mg./Kg.) on the cat's pressor and nictitating membrane responses to tyramine (500 mcg./Kg.) and cyclohexylamine (5 mg./Kg.) in a reserpinized (1 mg./Kg. i.p.) cat.

Effect of Cyclohexylamine After Pretreatment with Pargyline—These experiments were undertaken to determine if potentiation of the action of cyclohexylamine would be observed following animal pretreatment with a monoamine oxidase inhibitor. It should be recalled that pretreatment of patients with monoamine oxidase inhibitors resulted in a hypertensive crisis when these patients ingested the sympathomimetic agent, tyramine, contained in various foods (3, 4).

Therefore, pargyline (25 mg./Kg.), a monoamine oxidase inhibitor, was administered i.p. to cats 24 hr. prior to the start of the experiments. As can be seen in Fig. 5, pargyline pretreatment had no effect on the action of cyclohexylamine on either blood pressure or nictitating membrane response. However, in contrast, pretreatment with the monoamine oxidase inhibitor potentiated the responses seen after tyramine (500 mcg./Kg.). It can be seen that after tyramine there was a potentiation in both the pressor and in the nictitating membrane responses when compared to control values. Data obtained from all experimental animals are presented in Table I. Thus, there did not appear to be any significant interaction between this monoamine oxidase inhibitor and cyclohexylamine. This is most likely due to the fact that cyclohexylamine, in contrast to tyramine, is only a very weak substrate for MAO (5).

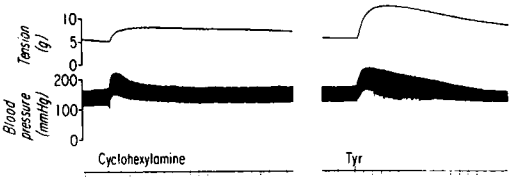

Fig. 5—The effect of cyclohexylamine (5 mg./Kg.) and tyramine (500 mcg./Kg.) on the pressor and nictitating membrane responses of a 24 hr. pargyline (25 mg./Kg. i.p.) pretreated cat.

TABLE I—CHANGE IN BLOOD PRESSURE[a] AND TENSION OF THE NICTITATING MEMBRANE IN CONTROL AND PARGYLINE-PRETREATED CATS

Experiment	Cyclohexylamine (5.0 mg./Kg.) Blood Pressure (mm. Hg)	Cyclohexylamine (5.0 mg./Kg.) Nictitating Membrane (Gm.)	Tyramine (500 mcg./Kg.) Blood Pressure (mm. Hg)	Tyramine (500 mcg./Kg.) Nictitating Membrane (Gm.)
Control	82 ± 8.2[b] (5)[c]	5.2 ± 0.62 (5)	64 ± 4.8 (7)	3.5 ± 0.71 (7)
Pargyline-pretreated (25 mg./Kg.)	73 ± 10.8 (6)	4.7 ± 0.98 (5)	78 ± 8.2[d] (6)	7.2 ± 0.52[d] (6)

[a] Blood pressure = diastolic pressure + 1/3 pulse pressure. [b] Mean value ± standard error of the mean. [c] Number of animals. [d] Significant difference from control ($p \leq 0.05$).

DISCUSSION

The data presented in this paper demonstrate that cyclohexylamine, a possible metabolite of cyclamate, possessed pressor activity and caused contraction of the nictitating membrane of the anesthetized cat. Cyclohexylamine had no effect upon the adrenergic, cholinergic, or histaminic responses elicited by epinephrine, norepinephrine, histamine, or acetylcholine.

The pressor activity of cyclohexylamine was shown to be due to the action of cyclohexylamine on the sympathetic nervous system. Prior administration of phenoxybenzamine, an irreversible competitive α-adrenergic receptor blocker, reversed the pressor response of cyclohexylamine and resulted in a depressor response which in turn was slightly blocked by the β-receptor blocking agent, propranolol.

Cyclohexylamine appeared to be an indirect acting sympathomimetic amine. After reserpine depletion of transmitter substance, cyclohexylamine was unable to produce its characteristic increase in blood pressure. The same result was seen with tyramine, which is a known indirect acting sympathomimetic amine. The responses to both tyramine and cyclohexylamine could be restored by slow administration of norepinephrine which has been shown to restore the depleted transmitter levels after reserpine pretreatment (6). Further evidence to indicate the indirect nature of cyclohexylamine was obtained by injecting cats repeatedly with the amine. After the first injection, the subsequent responses became progressively less until cyclohexylamine had only minimal effect. The same decrease in response has been demonstrated for tyramine.

Cyclohexylamine appeared to be like tyramine in many ways: (*a*) both produced hypertension in cats after i.v. injection, (*b*) both appeared to act indirectly by release of catecholamines, and (*c*) both were effective only after relatively high doses were administered. Recently, it was demonstrated that clinically useful monoamine oxidase inhibitors could potentiate or change the action of several other drugs and even certain foods (3, 4). For example, tyramine evoked, augmented, and prolonged hypertensive effects in animals and patients treated with inhibitors of monoamine oxidase. Usually innocuous amounts of tyramine present in certain food, including cheese caused hypertensive reactions in patients treated with the enzyme inhibitor.

Because cyclohexylamine produced a pressor response which closely resembled the pressor action of tyramine, and cyclohexylamine may possibly be ingested in many diets or produced from ingested substrate, its interaction with monoamine oxidase inhibitors was investigated. The action of cyclohexylamine was not potentiated in animals treated before with a monoamine oxidase inhibitor. This finding was in contrast to that demonstrated when tyramine was administered to animals pretreated with a monoamine oxidase inhibitor. After inhibition of this enzyme system the response to tyramine was potentiated and prolonged. The explanation for this dichotomy most likely is the fact that cyclohexylamine is not a substrate for monoamine oxidase; in fact, cyclohexylamine has been shown to be a weak inhibitor of the enzyme (5).

The possible clinical implications of these findings must wait until the metabolism and possible spontaneous breakdown of cyclamate are better understood. If an enzyme system is responsible for the hydrolysis of the artificial sweetening agent, then the possible substrate-induction of the enzyme must be considered. This, of course, could increase tremendously the amount of cyclohexylamine produced in the body and therefore also increase the possible toxicologic implications of the metabolite. Other pharmacological aspects concerning the interaction of the artificial sweetening agents and their metabolites are presently under investigation.

REFERENCES

(1) Kojima, S., and Ichibagase, H., *Chem. Pharm. Bull.*, **14**, 971(1963).
(2) Leahy, J. S., Wakefield, M., and Taylor, T., *B.I.B.R.A. Information Bull.*, **5**, 669(1966).
(3) Blackwell, B., and Mabbitt, L. A., *Lancet*, **1**, 938(1965).
(4) Blackwell, B., and Marley, E., *Brit. J. Pharmacol*, **26**, 120(1966).
(5) Sarkar, S., Banerjee, R., Ise, M. S., and Zeller, E. A., *Helv. Chim. Acta*, **43**, 439(1960).
(6) Burn, J. H., and Rand, M. J., *J. Physiol., London*, **144**, 314(1958).

Keyphrases

Cyclohexylamine—sympathomimetic activity
Pressor activity—cyclohexylamine
Phenoxybenzamine effect—cyclohexylamine activity
Reserpine effect—cyclohexylamine activity
Pargyline effect—cyclohexylamine activity