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SHORT COMMUNICATIONS

Lycorine inhibition of drug metabolism and ascorbic acid biosynthesis in the rat

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MANY compounds which stimulate drug metabolism in rats also enhance the urinary excretion of ascorbic acid[1] which represents an accelerated hepatic biosynthesis[2]. The finding that urinary ascorbic acid excretion is much greater than control values at times when drug metabolism is increased has led to the question of whether a causal relationship may exist between increased ascorbic acid excretion and increased hepatic microsomal drug metabolism.[3] However, there appears to be some discrepancy between the time course of stimulated drug metabolism and stimulated ascorbic acid biosynthesis.[4]

The ability of the alkaloid lycorine to decrease ascorbic acid excretion in control rats[5] and to partially block the stimulated excretion of the vitamin in chloretone-treated rats[6] suggests a possible means for examining the relationship between drug metabolism and ascorbic acid synthesis. The purpose of this investigation was to compare the properties of lycorine with those of the well-characterized inhibitor of drug metabolism, β-diethylaminoethyl 2,2-diphenylpentanoate (SKF 525-A), with respect to inhibition of drug metabolism and ascorbic acid biosynthesis. The ability of lycorine to modify the phenobarbital-induced stimulation of hepatic drug metabolism was also investigated.

EXPERIMENTAL METHODS

Male Holtzman rats weighing between 160 and 220 g were used in all experiments and were maintained on commercial laboratory chow with free access to water. Livers from each treatment group (4–6 animals) were pooled and homogenates prepared in 3 volumes of 0·1 M phosphate buffer (pH 7·4). The supernatant fraction resulting from centrifugation of such homogenates at 9,000 *g* was used in experiments *in vitro*. Enzymatic N-demethylation of butynamine [3-methyl-3-(N-methyl-t-butylamine)-butyne-1] was followed as a correlate of hepatic drug-metabolizing activity and was determined by measuring the amount of formaldehyde formed.[7] Ascorbic acid biosynthesis was measured with D-glucuronolactone as the substrate by a modification of the procedure of ul Hassan and Lehninger.[8]

The duration of barbiturate or meprobamate hypnosis was employed as an indicator of drug metabolism *in vivo*. Barbiturate and meprobamate sleep times were taken as that period of time when the animal was unable to right itself from a supine position when stimulated.

In experiments involving repeated lycorine administration, animals received five doses (5 mg/kg) over a two-day period. Three doses of lycorine or an equal volume of saline were given the first day, 6 hr apart; 12 and 24 hr after the third dose, lycorine or saline was again administered. All drugs were administered by i.p. injection.

RESULTS AND DISCUSSION

Lycorine and SKF 525-A caused a 50 per cent inhibition of butynamine N-demethylation when added to rat liver homogenates in concentrations of $2{\cdot}5 \times 10^{-3}$ M and $3{\cdot}5 \times 10^{-6}$ M respectively. The biosynthesis of ascorbic acid from D-glucuronolactone was inhibited 50% *in vitro* by $3{\cdot}5 \times 10^{-3}$ M lycorine, while SKF 525-A concentrations as high as $7{\cdot}5 \times 10^{-3}$ M had no effect.

Butynamine demethylation by rat liver homogenates was almost completely blocked 1 hr after i.p. injection of SKF 525-A but was inhibited only 40 per cent 1 hr after injection of lycorine (Table 1).

Data in Table 2 show that a single dose of lycorine causes a significant increase in the duration of hexobarbital, pentobarbital, and meprobamate hypnosis.

Barbital sleep times were also prolonged after lycorine pretreatment, suggesting that lycorine may prolong drug action by other mechanisms in addition to inhibition of drug metabolism.

Repeated administration of lycorine caused a 78 per cent inhibition of butynamine demethylation and a 41 per cent inhibition of ascorbic acid biosynthesis (Table 3).

TABLE 1. EFFECT OF LYCORINE AND SKF 525-A PRETREATMENT ON BUTYNAMINE METABOLISM*

Treatment	Dose (mg/kg)	Formaldehyde formed/g liver/hr† (μ moles)	Per cent of control
Saline	—	2·25	100·0
SKF 525-A	25	0·05	2·2
Lycorine	25	1·35	60·0

* Four animals per group received saline, SKF 525-A, or lycorine. One hour after injection, the animals were sacrificed and formaldehyde formation measured in homogenates prepared from pooled livers.

† Incubations were carried out in 25-ml Erlenmeyer flasks containing 30 μmoles glucose-6-phosphate, 50 μmoles semicarbazide, 50 μmoles nicotinamide, 50 μmoles magnesium chloride, 4 μmoles NADP, 1·0 ml 0·1 M phosphate buffer (pH 7·4), 1·0 ml of 9,000-*g* supernatant fraction (250 mg liver equivalent), and butynamine (10 μmoles) in a total volume of 6·0 ml. Flasks were incubated for 1 hr at 37° under oxygen. Formaldehyde was determined by the method of Cochin and Axelrod[9].

TABLE 2. EFFECT OF LYCORINE PRETREATMENT ON DURATION OF ACTION OF HYPNOTIC DRUGS*

Hypnotic	Duration of hypnosis (min)	
	Control	Lycorine
Hexobarbital	29 ± 7 (7)	76 ± 14 (7)†
Pentobarbital	147 ± 8 (5)	221 ± 21 (5)‡
Meprobamate	183 ± 8 (5)	294 ± 29 (5)‡
Barbital	401 ± 34 (7)	>667 (7)

* Lycorine (25 mg/kg) or saline (5 ml/kg) was administered 1 hr prior to hexobarbital Na (100 mg/kg), pentobarbital Na (35 mg/kg), meprobamate (300 mg/kg, suspended in 5% Tween 81), or barbital Na (250 mg/kg). In another group of animals the administration of 25 mg/kg SKF 525-A 1 hr preceding hexobarbital increased the sleep time to 172 ± 38 min. Values are plus or minus S.E; number of rats in parentheses.

† $P < 0.02$.

‡ $P < 0.005$.

TABLE 3. EFFECT OF REPEATED ADMINISTRATION OF LYCORINE ON BUTYNAMINE DEMETHYLATION AND ASCORBIC ACID BIOSYNTHESIS*

Treatment	Dose (mg/kg)	Formaldehyde formed/g liver/hr (μmoles)	Ascorbic acid formed/g liver/2 hr† (μmoles)
Saline	—	2·30	0·86
Lycorine	5	0·50	0·51

* Formaldehyde or ascorbic acid formation was measured in liver homogenates 18 hr after the last of five injections of lycorine or saline as described under experimental methods. Each group contained 4 animals.

† Ascorbic acid biosynthesis was measured in 25-ml Erlenmeyer flasks containing 1·0 ml of 9,000-*g* supernatant fraction (250 mg liver equivalent), 1·0 ml (40 μmoles) D-glucuronolactone, 1·0 ml 0·1 M phosphate buffer (pH 7·4), and distilled water to a total volume of 6·0 ml. Incubations were carried out for 2 hr at 37° under oxygen. "Total" ascorbic acid content of the flasks was measured by the method of Roe and Kuether[10].

Butynamine demethylation was increased four-fold 48 hr after a single injection of phenobarbital. When lycorine was administered according to the dosage regimen previously described and phenobarbital was administered concurrently, butynamine demethylation was enhanced to the same extent as in animals receiving phenobarbital alone (Table 4). In preliminary experiments it was not possible to obtain consistent and significant lycorine olockage of ascorbic acid excretion in the phenobarbital-treated rat.

TABLE 4. EFFECT OF LYCORINE ON PHENOBARBITAL-INDUCED ENHANCEMENT OF BUTYNAMINE DEMETHYLATION*

Treatment	Dose (mg/kg)	Formaldehyde formed/g liver/hr (μmoles)	Per cent of saline control
Saline	—	0·96	100
Lycorine	5	0·35	36
Phenobarbital	100	4·20	430
Phenobarbital†	100		
+	+		
Lycorine	5	3·89	400

* Formaldehyde formation was measured as in Table 1 in liver homogenates 48 hr after the injection of phenobarbital. Each group contained 4 animals.

† Lycorine was administered as described in Experimental Methods and phenobarbital was administered with the second dose of the alkaloid.

The data presented indicate that lycorine is a less potent inhibitor of drug metabolism than is SKF 525-A and, in addition, prolongs the hypnotic action of barbiturates by a mechanism(s) in addition to inhibition of drug metabolism.

Lycorine inhibits drug metabolism and ascorbic acid biosynthesis when added to liver homogenates or when using homogenates from pretreated rats. While these data do not directly support the existence of a role for ascorbic acid in drug metabolism, the fact that lycorine inhibits both butynamine N-demethylation and ascorbic acid biosynthesis at approximately equal concentrations must be further examined. Phenobarbital, a known stimulator of drug metabolism and ascorbic acid biosynthesis in the rat, has the ability to reverse the effect of repeated lycorine treatment on butynamine N-demethylation. These results suggest that it may be premature to dissociate ascorbic acid and hepatic drug metabolism.

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