Biochemical Pharmacology, 1962, Vol. 11, pp. 871–880. Pergamon Press Ltd., Printed in Great Britain.

EFFECTS OF BENZQUINAMIDE ON AVOIDANCE BEHAVIOR AND BRAIN AMINE LEVELS

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(*Received* 6 *April* 1962; *accepted* 31 *May* 1962)

Abstract—Benzquinamide, the acetate of 2-hydroxy-3-diethylcarbamyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11bH-benzoquinolizine, interferes with conditioned avoidance behavior in rats and monkeys. After doses of benzquinamide that reliably disrupt avoidance behavior, concentrations of noradrenaline (NA) and serotonin (5-HT) in the brain are within normal limits, and overt sedative or parasympathetic symptoms do not occur. Moreover, benzquinamide is only mildly susceptible to antagonism by monoamine oxidase (MAO) inhibition.

The high specificity of behavioral activity demonstrated by benzquinamide contrasts with the nonspecific effects of its parent alcohol: the alcohol is a potent depletor of brain NA and 5-HT, highly susceptible to reversal by an MAO inhibitor, and active in producing reserpine-like symptoms.

The rapid and potent effect of benzquinamide on avoidance behavior leads to the conclusion that its avoidance-disrupting activity is mediated by a mechanism other than depletion of NA and 5-HT from the brain.

CERTAIN RAUWOLFIA alkaloids (*e.g.* reserpine) and benzoquinolizine derivatives (*e.g.* tetrabenazine) reduce concentrations of noradrenaline and serotonin in the brain.[1–3] Concomitant with the lowered brain amine levels, these compounds produce a characteristic "reserpine syndrome"; in rats, this includes sedation, ptosis, miosis, and general reduction in sympathetic tone. The biological effects of reserpine mirror the time course of depleted amine levels, and not drug levels, in tissues, suggesting that depleted amine levels are responsible for the biological effects.[4] Other evidence also indicates that reduced central amine concentrations are responsible for the reserpine syndrome. For example, after pretreatment with monoamine oxidase inhibitors, which prevent the enzymatic inactivation of released central amines, appropriate doses of reserpine or tetrabenazine cause alertness, hyperactivity, exophthalmia, and mydriasis. Studies in these areas have recently been reviewed by Burns and Shore.[5]

During the course of establishing structure-activity relationships in the benzoquinolizine series a compound was encountered that exhibited an unexpected combination of behavioral and biochemical effects. It is the purpose of this paper to report certain quantitative findings from this agent, benzquinamide (Fig. 1), and to contrast its effects with those of its parent alcohol, a compound bearing a closer pharmacological resemblance to known active benzoquinolizines such as tetrabenazine.

METHODS

Materials

Freshly prepared suspensions of benzquinamide, the acetate of 2-hydroxy-3-diethylcarbamyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11bH-benzoquinolizine (Fig. 1), its parent alcohol (P-2565, Fig. 1), and nialamide[6] were administered to rats. The free bases were ground into fine aqueous suspensions and were administered at a constant volume of 5 ml/kg body weight. Physiological saline was administered at the same volume. The experimental compounds and tetrabenazine were administered orally (capsule) as the free bases to monkeys; empty capsules were given to controls.

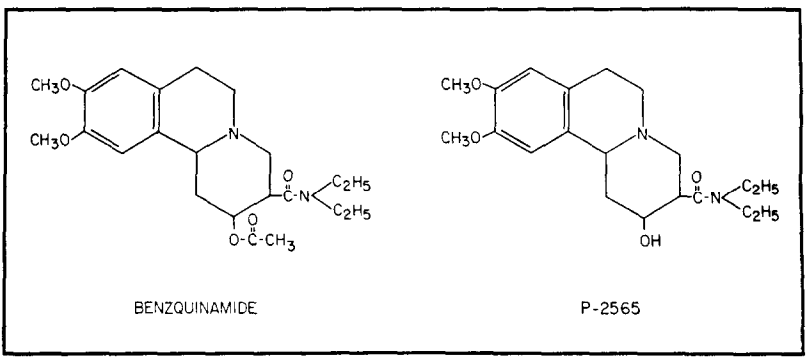

FIG. 1. Structural formulas of benzquinamide and its parent alcohol, P-2565.

Avoidance studies: rats

Modified Sidman[7] shock-avoidance schedules were used. Eight male Sprague-Dawley rats were trained in Lehigh Valley automatic multiple Skinner boxes to respond by pressing a lever to forestall 1 sec, 110 V, 1 mA electric shocks. Each response forestalled a shock for 40 sec; only 20 sec elapsed between shocks if no intervening responses were emitted. Sessions were 4 hr with rest periods of 28 hr. Behavior was stable and most shocks were successfully avoided after 3 months of training. Performance of a large sample of rats on this avoidance schedule has recently been described.[8] After training, 5 and 15 mg benzquinamide and P-2565/kg, and physiological saline were administered in random sequence to the eight rats. Treatments were spaced 4 days apart, and were administered 25–30 min after avoidance sessions began. This experiment was subsequently repeated in the same subjects except that each treatment followed a 24-hr pretreatment with an MAO inhibitor, nialamide (30 mg/kg intraperitoneally), and treatments were spaced 8 days apart.

Amine depletion studies: rats

Male Sprague-Dawley rats were drug-treated as described in the behavioral studies. At various times after drug administration groups of 8 rats were observed carefully for symptoms, and then sacrificed by decapitation; the brains were removed and immediately frozen at −20 °C. The brains were later assayed for NA and 5-HT by a spectrofluorimetric technique.[9] Homogenized brain tissue was pre-extracted twice with chloroform to remove any drug present, because benzoquinolizines interfere with the fluorimetric assay. Four determinations, each from two pooled rat brains, were performed at each time interval.

Metabolic studies in vivo*: rats*

Extracts of rat brain and liver tissue were prepared for paper chromatography by homogenizing the tissues in phosphate buffer (pH 10) and extracting with 6 volumes of *n*-heptane. The heptane extracts were concentrated under vacuum. Tissues from four rats were pooled for each sample. The concentrate from each sample was spotted on Whatman no. 4 paper impregnated with formamide-methanol (40 : 60) and submitted to descending chromatography utilizing carbon tetrachloride-diethylamine (9 : 1) as the mobile phase. The mobile phase was saturated with formamide. The compounds and their relative positions were identified by their fluorescence under ultraviolet light.

Avoidance studies: monkeys

Five mature rhesus monkeys (approximately 5 kg) were avoidance-trained in Foringer restraining chairs. Responses on an easily accessible Foringer lever forestalled 0·75 sec, 400 V, 5 mA electric shocks for 10 sec. The interval between shocks, when no responses were emitted, was also 10 sec. After training was completed, various doses of the benzoquinolizines were administered orally 30 min before the 5-hr sessions began. The order of treatments was mixed. At least two sessions of normal behavior elapsed between treatments. If a monkey received five shocks within a 1-min period (this happened only after some drug treatments) a 30-min rest period was allowed in order to protect the animal. The monkeys were observed for symptomatic changes 1·5 hr after drug administration. Observations were made through one-way vision glass, since direct examination interfered with the usual course of drug effects; therefore, estimates of only gross symptoms were made.

Amine depletion studies: monkeys

Benzquinamide and its parent alcohol were given to young rhesus monkeys (approximately 1·5 kg) at doses of 5 and 20 mg/kg orally (capsule). The monkeys were examined for symptoms 60–70 min after drugs and then sacrificed with intravenous pentobarbital. The brain stems were removed and analyzed for NA and 5-HT according to the methods used in rats. Duplicate analyses were conducted on each brain stem.

RESULTS

Avoidance studies: rats

Both doses of benzquinamide and its parent alcohol produced increased mean shock rates signifying disruption of avoidance behavior (Fig. 2, left). The maximal mean shock rate reached during any 10-min period after each treatment was selected from Fig. 2, left, and compared, by means of a *t*-test for paired samples,[10] with 10-min maxima from every other treatment. Maximal mean shock rates after 15 mg benzquinamide and P-2565/kg were significantly ($P < 0{\cdot}01$) higher than maximal mean shock rates after 5 mg of each drug/kg. Corresponding doses of benzquinamide and P-2565 produced 10-min mean shock rate maxima that were not significantly different from each other ($P > 0{\cdot}05$). Over-all mean shock rates for the 3 hr following drug administration, reflecting duration of action, were also compared. Over-all shock rates after both 15 mg/kg treatments were again significantly higher than those after 5 mg/kg treatments ($P < 0{\cdot}01$). Benzquinamide at 15 mg/kg caused a higher over-all mean shock rate for the 3 hr following dosage than did the alcohol at the same dose

($P < 0{\cdot}01$); over-all shock rates after the two 5 mg/kg doses were not significantly different ($P > 0{\cdot}05$) from each other.

These results were interpreted as showing that benzquinamide and its parent alcohol were approximately equipotent in their disruptive effects on avoidance, but that benzquinamide had a longer duration of action than its parent alcohol.

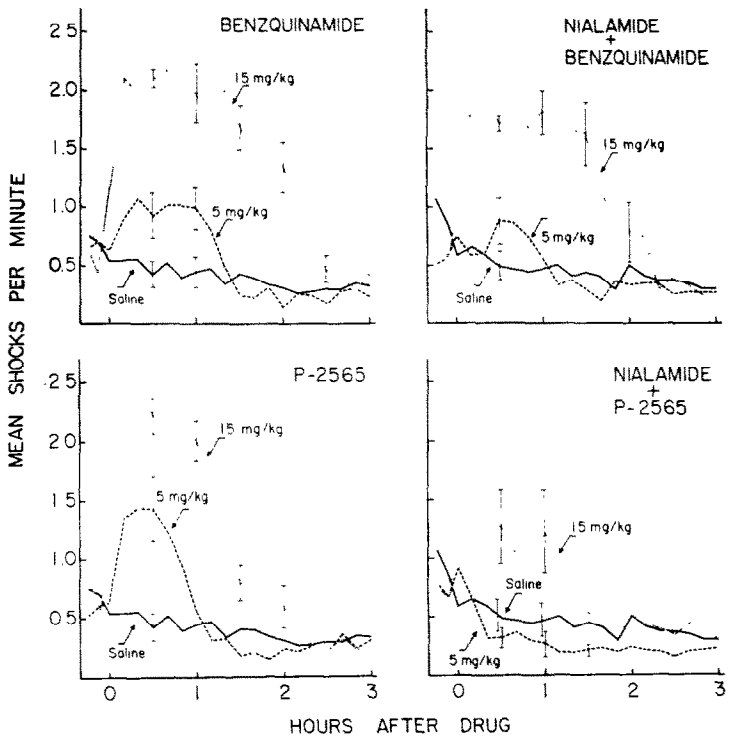

FIG. 2. Mean shock rates of 8 rats as a function of time after drug. Curves on the left were derived from an experiment in which each rat received 2 intraperitoneal doses of benzquinamide and P-2565 and physiological saline in random sequence. Curves on the right are from a similar, subsequent experiment in which each of the above treatments followed a 24-hr pretreatment with 30 mg of nialamide/kg, given intraperitoneally. Means were calculated for every 10-min period; representative standard errors of the means are illustrated as vertical bars.

The avoidance-disrupting effects of benzquinamide and P-2565 were attentuated by pretreatment with an MAO inhibitor, nialamide, but to different degrees (Fig. 2, right). For example, after nialamide pretreatment followed by 5 mg P-2565/kg, mean shock rates were significantly ($P < 0{\cdot}05$) *less* than were nialamide-control values from 30 to 60 min after administration of P-2565. Benzquinamide, on the other hand, continued to cause mean shock rates significantly ($P < 0{\cdot}01$) higher than control shock rates from 30 to 60 min after treatment. Point-by-point *t*-test comparisons showed that after nialamide pretreatment the disruptive effect of benzquinamide at both doses was reliably greater than that of P-2565. Nevertheless, the magnitude and especially the duration of disruption caused by benzquinamide at each dose (Fig. 2,

top left) appeared to be lessened somewhat by nialamide pretreatment (Fig. 2, top right).

Amine depletion studies: rats

The effects of benzquinamide and P-2565 on NA and 5-HT levels of brain in rats are shown in Fig. 3. P-2565 effectively and rapidly depleted the brain stores of these

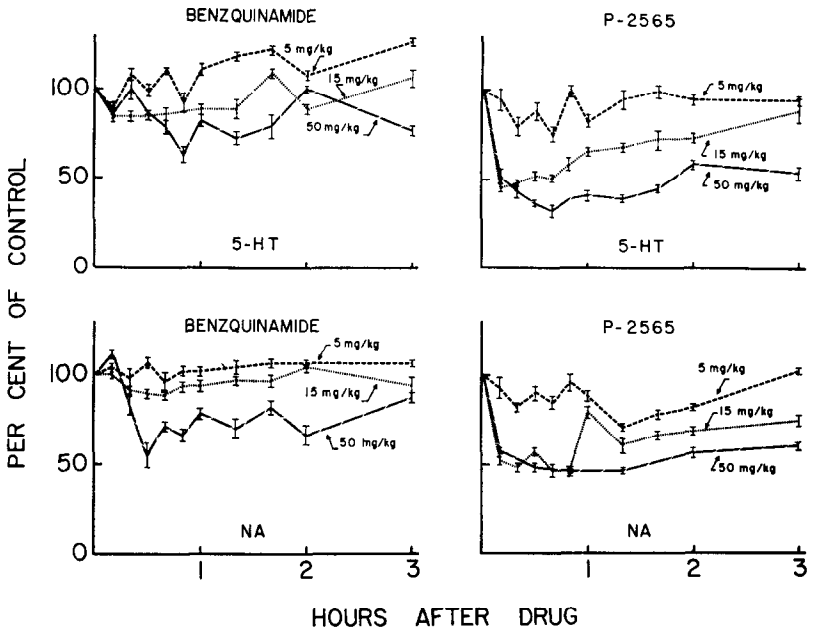

FIG. 3. Mean relative concentrations of NA and 5-HT in brain as a function of time after drug. Rats were treated with 3 intraperitoneal doses of benzquinamide or P-2565. They were sacrificed at various times afterward in groups of 8. Vertical bars represent standard errors of the means. Control values, in μg/g were as follows: NA, 0·49 $\pm$ 0·03; 5-HT, 0·46 $\pm$ 0·02.

amines. The magnitude and duration of its action in this regard were dependent upon the dose administered, as in the behavioral tests. Benzquinamide, on the other hand, at the same doses producing pronounced effects on avoidance behavior in the rat (5 and 15 mg/kg) did not cause a significant depletion of brain amine stores. When a higher dose of benzquinamide (50 mg/kg) was administered, some depletion of both NA and 5-HT was observed. The depletion of amines observed after benzquinamide treatment did not occur until about 20 min elapsed, in contrast with the very rapid depletion observed when P-2565 was administered. Moreover, it did not reach the 50 per cent-depletion level.

Benzquinamide, at a dose of 15 mg/kg, failed to produce the reserpine syndrome. When the dose was increased to 50 mg/kg, mild sedation and ptosis were observed. After the administration of 50 mg P-2565/kg, the rats showed a characteristic intense reserpine syndrome—*i.e.* salivation, lacrimation, diarrhea, miosis, ptosis, and deep sedation. The onset and duration of these symptoms very closely paralleled the onset and duration of amine depletion. Lower doses of P-2565 produced similar effects, but

to a lesser degree. It was noted that the reserpine-like symptoms at 15 mg P-2565/kg were more pronounced than were those after 50 mg benzquinamide/kg.

Metabolic studies in vivo*: rats*

Chromatographic analysis of the brain tissue of those rats receiving benzquinamide revealed that the drug was partially converted to the free alcohol *in vivo*. These results are summarized in Table 1.

TABLE 1. PRESENCE OF ADMINISTERED BENZQUINAMIDE AND P-2565 IN RAT BRAIN AND LIVER

Time after drug*	Drug (50 mg/kg i.p.)	Tissue	Drug presence† Benz.	P-2565
0	None	Brain	—	—
	None	Liver	—	—
10	Benz.	Brain	+	±
	Benz.	Liver	±	+
20	Benz.	Brain	+	+
	Benz.	Liver	±	+
30	Benz.	Brain	±	+
	Benz.	Liver	±	+
60	Benz.	Brain	+	—
	Benz.	Liver	±	±
20	P-2565	Brain	—	+
	P-2565	Liver	—	+

* Time of sacrifice after drug administration in minutes.
† + = present; — = absent; ± = very weak fluorescent area.

TABLE 2. INCIDENCE OF AVOIDANCE DISRUPTIONS* IN MONKEYS TREATED WITH BENZQUINAMIDE, P-2565, AND TETRABENAZINE

Drug (capsules)	Dose (mg/kg p.o.)	No. of disruptions* / No. of experiments	Approximate ED_{50} (mg/kg p.o.)
Empty capsule		0/44	
Benzquinamide	0·5	0/2	<1, >0·5
	1	5/5	
	2	2/2	
	3	3/3	
	4	2/2	
	5	5/5	
P-2565	0·5	0/2	<1, >0·5
	1	2/2	
	3	2/2	
	4	1/2	
	5	5/5	
Tetrabenazine	1	0/2	>10
	5	2/5	
	10	2/5	

Data reported are from a group of 5 mature rhesus monkeys. Different monkeys were used within each dose level; thus, when the denominator is 5, each monkey was subjected to the dose once. The two monkeys used at 1 and 5 mg tetrabenzine/kg were the subjects for which activity was observed at 10 mg/kg.

* Criterion of disruption was 5 or more shocks received during the experimental session.

Avoidance studies: monkeys

Benzquinamide consistently disrupted avoidance behavior in monkeys at 1 mg/kg and above (Table 2). Onset of drug effect at all active doses was within 1 hr, and duration of effect was dependent on dose (*e.g.* Fig. 4). Peak action occurred 1–1·5 hr after drug administration. The alcohol was similar to benzquinamide in its avoidance-disrupting potency; its duration of effect tended to be relatively short-lived. Tetrabenazine was less potent in monkeys than either benzquinamide or P-2565.

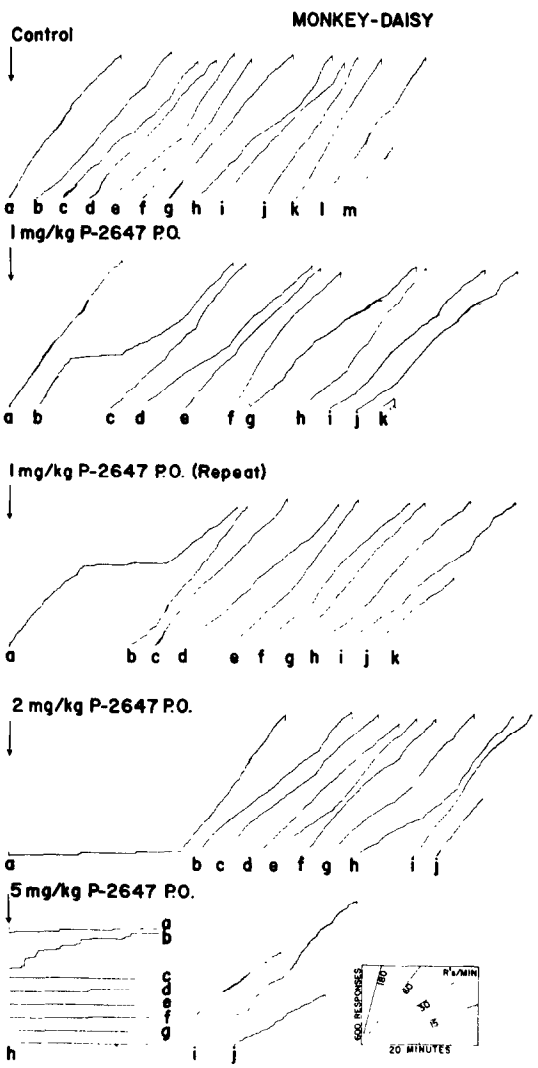

FIG. 4. Cumulative response curves in a monkey, illustrating the dose-effect relationships of benzquinamide on avoidance behavior. Drugs were administered orally 30 min before the beginning of each session shown. Successive curve segments, labeled with letters, were condensed to save space. Shocks are not shown.

Except for two experiments at 5 mg/kg, benzquinamide failed to elicit gross symptoms in the avoidance-trained monkeys. Symptoms during the two experiments in question resembled those observed after all active (Table 2) 4 and 5 mg doses of P-2565/kg, and after all active doses of tetrabenazine: a stuporous, catatonic state with very pronounced ptosis and diarrhea.

Amine depletion studies: monkeys

Benzquinamide did not deplete the NA and 5-HT stores in brain of the monkey after oral doses of 5 and 20 mg/kg. The analytical data are shown in Table 3. Similarly,

TABLE 3. BRAIN AMINE LEVELS AFTER ADMINISTRATION OF BENZQUINAMIDE AND P-2565 TO MONKEYS*

Drug (capsule)	Dose (mg/kg p.o.)	Determinations	Brain amine levels (μg/g) NA	5-HT
Control		4	0·57–0·79	0·43–0·53
Benzquinamide	5	2	0·61, 0·61	0·43, 0·45
	20	2	0·68, 0·71	0·57, 0·63
P-2565	5	2	0·50, 0·50	0·47, 0·49
	20	2	0·54, 0·54	0·49, 0·53

* Monkeys were sacrificed 1–1·5 hr after drug administration.

no significant reduction in concentrations of brain NA or 5-HT occurred after P-2565 treatment under the conditions of this experiment.

All the monkeys, except for the one receiving 20 mg P-2565/kg, were asymptomatic just before sacrifice. The exceptional subject was mildly sedated, with some ptosis, miosis, and diarrhea in evidence.

DISCUSSION

The present data indicate that the disruption of avoidance behavior evoked by benzoquinolizine derivatives does not necessarily go hand in hand with lowered concentrations of NA and 5-HT in brain. In support of this conclusion are the following findings: (a) in rats, doses of benzquinamide that evoked no depletion of NA and 5-HT from brain tissue were nevertheless active in disrupting conditioned avoidance; (b) despite the very different potencies of benzquinamide and P-2565 as depletors of NA and 5-HT stores in the rat brain, both compounds were approximately equipotent in disrupting avoidance behavior; (c) doses of benzquinamide and P-2565 that failed to deplete NA and 5-HT from the brain stem of monkeys were twenty times larger than doses that disrupted avoidance behavior in monkeys.

Previous work indicates that sedative action, parasympathetic symptoms, and susceptibility to antagonism by MAO inhibition are correlated, among benzoquinolizine derivatives, with amine-depleting activity.[7] If the disruption of conditioned avoidance behavior is omitted from consideration, the present data do not contradict this correlation. In both rats and monkeys P-2565 was more potent than benzquinamide in inducing such symptoms as sedation, ptosis, lacrimation, salivation, and miosis. Moreover, nialamide, a known MAO inhibitor, antagonized the avoidance-disrupting

activity of P-2565 more than that of benzquinamide in rats. These findings correlate with the far greater potency of P-2565, compared with benzquinamide, in depleting brain NA and 5-HT in this species.

Although disruption of avoidance behavior occurred without reserpine-like side effects after low doses of benzquinamide, after treatment with a high dose (50 mg/kg) of benzquinamide relatively weak and delayed amine depletion, sedation, and parasympathetic symptoms did occur in rats. The chromatographic data suggest that deacetylation *in vivo* of benzquinamide to its parent alcohol occurs in the rat. The metabolite (P-2565) may account for the mild reserpine syndrome after the high dose of benzquinamide. Other findings further support this possibility: the onset of amine depletion caused by benzquinamide was delayed at 50 mg/kg, in contrast with the rapid onset seen after P-2565, possibly denoting the time required for deacetylation. Furthermore, the duration of action of benzquinamide on avoidance was longer than that of the free alcohol. Residual activity caused by metabolite-induced amine depletion might cause such a prolongation, a supposition deriving support from the prominent antagonism by nialamide of the terminal segment of avoidance disruption caused by benzquinamide. In contrast, the rapid onset and high potency of benzquinamide with respect to avoidance behavior indicate that, despite its conversion to its free alcohol at high doses, benzquinamide is itself a potent agent that acts rapidly.

The fact that depressant symptoms in the avoidance-trained monkeys were more pronounced and occurred at lower doses than in the monkeys examined for amine concentrations has several possible explanations: *e.g.* (a) younger monkeys were used in the biochemical studies; (b) conditions of restraint were different in the two studies; (c) a single, fairly short time before sacrifice, based on the behavioral data, was selected in the biochemical experiments. These variations between experiments dampen somewhat interpretations of the combined monkey data with respect to avoidance and amine effects.

It is noteworthy, however, that benzquinamide was more potent than tetrabenazine in disrupting avoidance behavior in monkeys. Previously reported avoidance experiments in rats using parameters similar to those in the present experiment[11] and preliminary studies in our laboratory both suggest that tetrabenazine is more potent than is benzquinamide in disrupting avoidance behavior in rats.

The enhanced relative potency of benzquinamide in monkeys in the present experiments may result from species specificity or from better oral absorption of benzquinamide. In any case, this enhanced potency in primates, combined with the interesting pharmacological profile of benzquinamide, has prompted clinical trials of the material as an anti-anxiety agent having specific action. Initial results from these trials are encouraging.[12–14]

Acknowledgements—Benzquinamide and P-2565 were synthesized by Dr. J. R. Tretter. Tetrabenazine was generously provided by Hoffman-La Roche, Inc. The assistance of Mr. M. Lynch in conducting the chromatographic experiment is gratefully acknowledged.

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