ANTICONVULSANT PROPERTIES OF SOME NEWER MONOAMINE OXIDASE INHIBITORS

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Facilitation of experimental convulsions by reserpine (1) which persisted for several days was found to correspond with depletion of brain amines including 5-hydroxytryptamine, adrenaline and noradrenaline. Decrease in the concentration of these amines was found to be responsible for such experimental convulsions. It has also been reported that inhibitors of the enzyme monoamine oxidase, responsible for the metabolism of biogenic amines, have pronounced anticonvulsant effect (2) presumably due to an increase in the concentration of brain amines. In the present study some quinazolone hydrazides (3) and quinazolone hydrazines (4) synthesized in this laboratory as monoamine oxidase inhibitors were tested for their ability to protect against convulsions produced in rats by subcutaneous injections of pentylenetetrazol. Attempts were made to investigate if the enzyme inhibition is related to anticonvulsant effects elicited by such 2,3-disubstituted quinazolones which have been shown to possess pronounced hypnotic (5) and anticonvulsant properties (6).

METHODS

The synthesis of newer 2,3-disubstituted quinazolones and their *in vitro* effect on the monoamine oxidase activity of isolated rat liver mitochondria using tyramine as substrate have been reported elsewhere (3, 4). In the present study *in vivo* inhibition of monoamine oxidase by quinazolone hydrazides and quinazolone hydrazines was determined by DOPA (3,4-dihydroxyphenylalanine) response test (7) and reserpine reversal test (8).

DOPA response test

Albino mice of either sex weighing between 15-20 g were used. The mice were divided in groups of five and kept in separate cages. A group of 5 mice was used for each dose of these agents. Aqueous solution of DL-DOPA was prepared by first dissolving the powder in 1 N HCl and then adjusting the pH with dilute sodium hydroxide to as close to neutral as possible (6.0) avoiding any precipitate. All injections were made within 1 hour after the preparation of the solution (7). Quinazolone hydrazides and

TABLE 1. Some newer monoamine oxidase inhibitors.

Chemical name	Structural formula	Referred as
1-Methyl phenpropyl hydrazine or phenyl isobutyl hydrazine		JB-835
β -Phenylethyl hydrazine (Nardil)		Phenelzine
N-Benzyl-N-methyl-2-propynyl- amine hydrochloride (Eutonyl)		Pargyline
2-Methyl hydrazino-3-phenyl-4- quinazolone		QZH-1
2-Ethyl hydrazino-3-phenyl-4- quinazolone		QZH-2
2-Methyl-3(2')-benzoyl hydrazino-4-quinazolone		QZH-3
2-Methyl-3(4')-benzoyl hydrazino-4-quinazolone		QZH-4
2-Methyl-3(4')-benzoyl hydrazino- 6-chloro-4-quinazolone		QZH-5
2-Methyl-3(4')-benzoyl hydrazino- 6-bromo-4-quinazolone		QZH-6
2-Methyl-3(4')-benzoyl hydrazino-6-iodo-4-quinazolone		QZH-7

quinazolone hydrazines were administered orally (150 mg/kg) in 5% aqueous suspension of gum acacia while JB-835, pargyline and phenelzine were given intraperitoneally in doses of 20 mg/kg. In the present study DL-DOPA (100 mg/kg) was administered intraperitoneally. Administration of DL-DOPA, in animals which were treated previously by monoamine oxidase inhibitors, produced piloerection, increased motor activity, irritability, tremors, jerks and the fighting reaction, being the maximal response. On the other hand animals receiving DOPA alone showed none of these symptoms at the dose tested. The compounds were considered inactive if none of the characteristic signs produced by DL-DOPA were present in the pretreated animals.

Reserpine reversal test

Albino mice of either sex weighing between 15 to 25 g were used. The mice were divided in groups of five and kept in separate cages. A group of 5 mice was used for each dose of the test compound.

Quinazolone hydrazides and quinazolone hydrazines were given orally in 5% aqueous suspension in gum acacia in a dose of 200 mg/kg. Reserpine was also administered intraperitoneally (5 mg/kg). In mice pretreated with monoamine oxidase inhibitors, reserpine no longer caused sedation, or miosis but instead caused excitation, piloerection, mydriasis and increased motor activity after one hour of administration. Failure to produce reversal of reserpine effect was interpreted as the drug being inactive.

Metrazol seizures threshold test

Anticonvulsant property was determined in rats of either sex weighing between 30 to 100 g (9). The rats were divided in groups of 10, keeping the group weights equal as far as possible. One group of ten animals was used for each dose of the compounds. Quinazolone derivatives were fed orally suspended in 5% aqueous gum acacia while JB-835, pargyline and phenelzine were administered intraperitoneally. Six hours after the administration of the monoamine oxidase inhibitors, pentylenetetrazol (Metrazol) was injected subcutaneously under the loose skin of the back in a dose of 80 mg/kg. This dose of pentylenetetrazol caused convulsions in almost all untreated normal animals. The animals were observed for the next 60 minutes for the occurrence of seizures. One episode of clonic spasm which persisted for at least a five second period was considered a threshold convulsion. Transient intermittent jerks or tremulousness were not taken into account. Animals not exhibiting even a threshold convulsion during the 60 minutes period were considered protected. The number of animals protected in each group was recorded and percentage protection was determined for each dose.

The various quinazolone hydrazides and quinazolone hydrazines used in the present study are recorded in Table 1. JB-835, pargyline and phenelzine, the well established monoamine oxidase inhibitors, were used as reference standards.

RESULTS

DOPA response test

Administration of DOPA in animals pretreated with JB-835, pargyline and phenel-

zine produced marked stimulation, piloerection, increased motor activity, irritability, tremors, jerks, straub tail, exophthalmos and fighting behaviour. The mortality in cases of JB-835 and phenelzine was 40% whereas with pargyline it was 100 per cent. With newer monoamine oxidase inhibitors only QZH-2, QZH-5 and QZH-7 were found to produce mild stimulation and slight increase in motor activity. Tremors and jerks were found in 20% mice with QZH-2 and QZH-5 and in 40% mice with QZH-7. All those

TABLE 2. Anticonvulsant properties of monoamine oxidase inhibitors.

Compound	<i>In vitro</i> monoamine oxidase inhibition (%)	Anticonvulsant activity		24 hr mortality (%)
		Dose (mg/kg)	Protection (%)	
JB-835 (3×10^{-6} M)	79	10	20	30
		20	30	50
		30	50	60
		50	10	90
Phenelzine (1×10^{-5} M)	78	10	20	20
		20	50	20
		30	50	60
		40	30	60
Pargyline (3×10^{-6} M)	78	10	20	30
		20	40	20
		30	50	30
		40	40	40
QZH-1 (1×10^{-4} M)	15	40	0	40
QZH-2 (1×10^{-4} M)	53	40	40	0
		80	60	0
		160	40	10
QZH-3 (1×10^{-4} M)	12	40	20	0
		80	60	0
		160	30	10
QZH-4 (1×10^{-4} M)	11.3	40	0	0
QZH-5 (1×10^{-4} M)	60.27	40	20	20
		80	50	20
		160	30	40
QZH-6 (1×10^{-4} M)	52.6	40	20	30
		80	50	20
		160	30	70
QZH-7 (1×10^{-4} M)	75.03	40	20	20
		80	30	20
		120	50	20
		160	30	30

Figures in parenthesis represent final concentration of the inhibitors.

Percentage inhibition was calculated from the decrease in the oxygen uptake (3, 4)

animals which showed some activity ultimately died. On the other hand slight stimulation without increase in activity or jerks was observed with QZH-3 and QZH-6 and all the animals survived. QZH-1 and QZH-4 were devoid of any DOPA potentiating effect.

Reserpine reversal test

Administration of reserpine in animals pretreated with JB-835, pargyline and phenelzine produced after 30–60 minutes, marked excitation, exophthalmos, piloerection and other sympathomimetic effects. QZH-3, QZH-5, QZH-7 produced only slight stimulation and no marked increase in activity or other sympathomimetic signs could be observed. On the other hand no reversal of reserpine effect could be observed in animals pretreated with QZH-1, QZH-2, QZH-4 and QZH-6.

Anticonvulsant activity

The anticonvulsant properties of JB-835, pargyline, phenelzine and newer monoamine oxidase inhibitors against pentylenetetrazol threshold test are shown in Table 2. The monoamine oxidase inhibitory properties of these compounds as reported earlier (3,4) are also included for the sake of discussion. Anticonvulsant properties of JB-835, pargyline and phenelzine were found to increase with simultaneous increase in the dose of these compounds. As shown in Table 2 in no instance more than 50% protection against pentylenetetrazol induced seizures could be obtained. Further increase in the dose was found to decrease the degree of such protection. Increase in the mortality rate within 24 hours was observed with JB-835 and pargyline and not with phenelzine. Amongst the newer monoamine oxidase inhibitors QZH-1 and QZH-4 were found to be devoid of anticonvulsant activity when used in doses of 40 mg/kg. On the other hand QZH-2, QZH-3, QZH-5, QZH-6 and QZH-7 were found to possess anticonvulsant properties. The degree of protection observed against pentylenetetrazol induced seizures was of the similar pattern as was found with known monoamine oxidase inhibitors. With all quinazalone derivatives maximum protection was observed in a dose of 80 mg/kg except QZH-7 where a dose 120 mg/kg was required to produce maximum protection of 50%. Increase in doses beyond these levels was found to decrease the degree of protection and caused simultaneous increase in mortality within 24 hours (Table 2). There seems to be a maximum effect of these known and as well as newer monoamine oxidase inhibitors and in no case total protection (100%) could be observed in the present study.

DISCUSSION

In the present study the *in vitro* enzyme inhibitory effects of JB-835, pargyline and phenelzine were found to correspond with the *in vivo* effects showing marked potentiation of DOPA response and reversal of the reserpine effects. The anticonvulsant property of these inhibitors as indicated by protection from pentylenetetrazol induced seizures was found to be unrelated to their monoamine oxidase inhibitory activity. Phenelzine, the comparatively less effective monoamine oxidase inhibitor was found to afford 50% protection when administered in a dose of 20 mg/kg while JB-835 and pargyline at this dose level showed 30% and 40% protection respectively. Contrary to the findings of Everett

et al. (7) we have found that pargyline in doses of 10–30 mg/kg showed anticonvulsant effect against pentylenetetrazol induced seizures in rats (Table 2). These investigators used pargyline in doses of 100–200 mg/kg and determined anticonvulsant properties after 1, 4 and 24 hours interval following drug administration in mice (10). It seems that the high dose of pargyline and species differentiation could account for such a difference. As is evident from Table 2, pargyline, JB-835 or phenelzine caused a peak in protection after which the protective ability falls inspite of further increase in the dose of the drug. Our results are in good agreement with those reported earlier (2) where central origin of the anticonvulsant effect of monoamine oxidase inhibitors has been demonstrated. Enzyme inhibitory effects of newer monoamine oxidase inhibitors could not be fully demonstrated with *in vivo* experiments. Mild stimulation of the DOPA response test and slight reversal of reserpine effects were observed with QZH-2, QZH-5 and QZH-7 presumably due to greater enzyme inhibitory properties as compared to QZH-1, QZH-3 and QZH-4. At present it is difficult to explain the failure of QZH-6 to exhibit positive DOPA response test and reserpine reversal test inspite of appreciable greater monoamine oxidase inhibitory properties comparable to that of QZH-2, QZH-5 and QZH-7. The anticonvulsant properties of newer monoamine oxidase inhibitors were found to follow a pattern similar to that observed with known inhibitors (JB-835, pargyline and phenelzine). The protection against pentylenetetrazol induced seizures observed with newer inhibitors was found to bear no relationship with their enzyme inhibitory properties. Increase in the dose of the inhibitor was found to produce greater protection upto a certain point. Further administration was found to decrease the degree of protection with simultaneous increase in mortality within 24 hours of administration. In the present study in no instance could total protection (100%) be observed either with the known as well as newer inhibitors. These results indicate that monoamine oxidase inhibition is presumably not the only mechanism responsible for the anticonvulsant properties of these compounds. Increase in the concentration of an unknown substance in addition to noradrenaline or 5-hydroxytryptamine (2) might presumably be concerned with anticonvulsant properties of these monoamine oxidase inhibitors.

SUMMARY

Some newer quinazalone hydrazides and quinazalone hydrazines having monoamine oxidase inhibitory properties were tested for their ability to protect against convulsions produced in rats by subcutaneous injections of pentylenetetrazol. *In vitro* inhibitory effects were demonstrated to some extent with *in vivo* studies as evidenced by potentiation of 3,4-dihydroxyphenylalanine (DOPA) response test and reversal of the reserpine effects. Attempts were made to investigate if anticonvulsant properties show correlation with their *in vitro* enzyme inhibition. It was found that the degree of protection offered by these compounds in no way parallel their enzyme inhibitory properties. Our results have indicated that monoamine oxidase inhibition is presumably not the only mechanism responsible for the anticonvulsant properties of the newer inhibitors.

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REFERENCES

- 1) CHEN, G., ENSOR, C.R. AND BOHNER, B.: *Proc. Soc. exp. Biol. Med.* **86**, 507 (1954)
- 2) PROCKOP, D.J., SHORE, P.A. AND BRODIE, B.B.: *Ann. N.Y. Acad. Sci.* **80**, 643 (1959)
- 3) PARMAR, S.S. AND ARORA, R.C.: *Can. J. Chem.* **44**, 2100 (1966)
- 4) PARMAR, S.S. AND ARORA, R.C.: (*In press*)
- 5) GUJRAL, M.L., KOHLI, R.P. AND SAXENA, P.N.: *Indian J. med. Sci.* **10**, 871 (1956)
- 6) GUJRAL, M.L., SAREEN, K.N. AND KOHLI, R.P.: *Indian J. med. Res.* **45**, 207 (1957)
- 7) EVERETT, G.M., WIEGAND, R.G. AND RINALDI, F.U.: *Ann. N.Y. Acad. Sci.* **107**, 1068 (1963)
- 8) CHESIN, M., KRAMER, E.R. AND SCOTT, C.C.: *J. Pharmac. exp. Ther.* **119**, 453 (1957)
- 9) SWINYARD, E.A., BROWN, W.C. AND GOODMAN, L.S.: *Ibid.* **106**, 319 (1952)
- 10) TOMAN, J.E.P., EVERETT, G.M. AND RICHARDS, R.K.: *Tex. Rep. Biol. Med.* **10**, 96 (1952)