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PHARMACOLOGICAL ACTIONS OF BERBERINE ON THE CENTRAL NERVOUS SYSTEM

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The alkaloid, berberine is widely distributed in the vegetable kingdom. The berberine containing plants are largely used in indigenous medicine. A crude extract known as "Rasaut, Rasvanti and Rasanjana" is a widely used household remedy as a stomachic, bitter tonic and diaphoretic. There is a renewed interest in berberine because of its reported usefulness in diarrhoeas (1) cholera (2) and experimental amoebiasis (3, 4). Some of our studies on the actions of berberine on the central nervous system are presented in this paper.

MATERIAL AND METHODS

Berberine was used in the form of its hydrochloride. For experiments on mice and rats, solutions were so prepared that not more than 0.2 ml of fluids was injected.

Behavioural effects in conscious cats: The effect of berberine administered by intraperitoneal route (i.p.) was studied in 9 conscious cats at 3 dose levels namely, 5, 20 and 40 mg/kg in a volume of 5 ml. The cats were observed continuously for first 3 hours and then at hourly intervals for the next 12 hours.

In 3 cats, berberine was administered by intraventricular route. Under pentobarbitone anaesthesia, a cannula was implanted in the lateral ventricle by the technique of Feldberg and Sherwood (5). The cats were allowed to recover and rested for 72 hours. After this period, the effect of berberine (100 μg) was studied in one cat and in doses of 1 mg each in 2 cats. The cats were observed continuously for 3 hours and then at intervals till they returned to normal. Two-tenth ml of berberine solution was used for injection. A half ml of 0.9% NaCl was used to wash the lumen of the cannula. At the end of each experiment, the position of the cannula was verified.

Effects in conscious mice: The motor activity of white mice was studied in a modified jiggle cage, the base of which rested on 4 springs and was connected to a frontal writing lever which wrote on a smoked drum. The effect of berberine (5 mg/kg i.p.) on amphetamine (4 mg/kg i.p.) treated mice was studied in 5 experiments.

Pentobarbitone sleeping time: Four groups of 10 albino mice each were administered pentobarbitone in doses of 25, 30, 35 and 40 mg/kg i.p. respectively. Sleeping time was calculated as the period from the loss of righting reflex till the time of recovery of righting reflex 3 times in a minute. After a rest period of 8 days, the same mice were treated with berberine (5 mg/kg i.p.) half an hour prior to injection of pentobarbitone and sleep-

ing time noted. Significance of results was calculated from duration of sleeping time using student 't' test.

One additional group of 10 mice was administered pentobarbitone (35 mg/kg i.p.) and those animals who lost righting reflex separated. When these mice regained the righting reflex, berberine (5 mg/kg i.p.) was injected to note whether animals again lost their righting reflex.

Amphetamine toxicity: Effect of berberine (5 mg/kg i.p.) on aggregate amphetamine (15 mg/kg i.p.) toxicity was studied in group of 10 male mice at room temperature of 25°C (6). Chlorpromazine (1 mg/kg i.p.) served as reference compound. Berberine or chlorpromazine was injected 30 minutes prior to amphetamine. The dead mice were removed from time to time and the total number of mice who survived in each group was noted at end of 20 hours.

The experiment was repeated in 2 groups of 10 mice kept singly in cages. One group was treated with amphetamine (100 mg/kg i.p.) and the other was pretreated with berberine 5 mg/kg i.p.

Leptazol seizures: 3 groups of 10 mice each were administered leptazol in doses of 20, 50 and 75 mg/kg i.p. respectively. The effect of berberine (5 mg/kg i.p.) on leptazol induced convulsions was studied in 3 other groups of 10 mice each, berberine being injected 30 minutes prior to leptazol. Criteria for convulsions was clonic convulsions for at least 10 seconds.

Supramaximal electroshock convulsions; Male mice, weighing 25 g, were administered a shock of 120 mA for 0.2 seconds through a pair of ear electrodes and only those who responded (tonic hind limb extension) were selected. Selection was done 48 hours prior to the actual experiment. The animals were allowed free access to food and water except during the actual experiment. The effect of berberine (5 mg/kg i.p.) on the supramaximal electroshock convulsions was studied in 10 mice. Ability to abolish the hind limb tonic extensor component of the maximal seizure pattern was taken as an index of anticonvulsant action of the drug. 10 mice treated with phenobarbital sodium (20 mg/kg i.p.) served as control.

Pain threshold: Effect of berberine (5 mg/kg i.p.) on pain threshold in untreated rats and rats treated with morphine (5 mg/kg i.p.) or pentobarbitone (15 mg/kg i.p.) was studied using the hot wire test as described by Gupta and Kulkarni (7). The experiment was performed in groups of 10 rats, each rat serving as its own control. Interval in between experiments was 8 days. Berberine was injected 30 minutes prior to morphine or pentobarbitone. Significance of results was calculated using the paired 't' test.

RESULTS

Effects in Cats

Berberine when administered by intraperitoneal route at all 3 dose levels studied namely 5, 20 and 40 mg/kg produced the following effects which commenced in 3-5 minutes-sedation, retching, urination and defaecation with straining. These effects

which were seen in 8 cats lasted for less than 2 hours. One cat which was administered berberine in a dose of 5 mg/kg showed a rage reaction which commenced 3 minutes after the injection and was most severe after 13 minutes. The cat returned to normal after an hour.

All the 3 cats who were administered berberine by the intraventricular route showed similar effects. The cats were sedated, inactive, disinterested and did not consume food. Effects attributable to peripheral cholinergic stimulation were conspicious by their absence. The effects appear to be dose dependant, being more intense in the cats who received 1 mg as compared to the one with 100 μg. Moreover, effects commenced immediately and lasted for 36 to 40 hours in the cats who were treated with 1 mg. With 100 μg berberine, effects commenced in 21 minutes and the cat recovered after 20 hours.

Effects in conscious mice

The spontaneous motor activity of white mice recorded in the modified jiggle cage is shown in Fig. 1. Administration of amphetamine (4 mg/kg i.p.) markedly increased the motor activity, this effect being maximum after 20 minutes. Administration of berberine (5 mg/kg i.p.) at peak activity of amphetamine, sedated the mice in 5 minutes, motor activity being reduced to even below the control level.

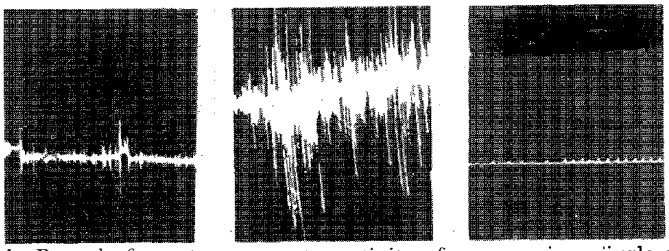

FIG. 1. Record of spontaneous motor activity of a mouse in a jiggle cage. A : Control. B : Amphetamine 4 mg/kg. C : 5 minutes after berberine 5 mg/kg administered at peak activity of amphetamine.

TABLE 1. Average sleeping time of pentobarbitone in mice in doses of 25, 30, 35 and 40 mg/kg i.p. and its modification after berberine 5 mg/kg i.p.

Pre-treatment	Average sleeping time (with error of mean in minutes) after pentobarbitone. Dose in mg/kg			
	25	30	35	40
Saline (Control)	25 (1)	32±12.6 (3)	33± 5.1 (6)	31 ±3.3 (8)
Berberine (5 mg/kg)	51 (2)	54±10.7 (5)	77±12* (8)	92.8±4.4* (10)

Number in parenthesis indicates number of animals sleeping in a group of 10 mice.

* indicates statistical significance $p<0.01$.

Pentobarbitone sleeping time

Table 1 shows the sleeping time of pentobarbitone before and after berberine (5 mg/kg i.p.). Though berberine potentiated the sleeping time at all 4 dose levels, statis-

tically significant differences were obtained only at the higher dose levels of pentobarbitone, namely 35 and 40 mg/kg. It is interesting to note that in the control animals, the sleeping time has not increased with increasing doses of pentobarbitone, although there is an increase in the number of animals losing righting reflex. However, in the animals pretreated with berberine, there is both an increase in sleeping time as well as in number of animals losing righting reflex with increasing dose of pentobarbitone.

Of the group of 10 mice who received 35 mg/kg i.p. of pentobarbitone, 6 lost the righting reflex. These animals on regaining the righting reflex received berberine 5 mg/kg i.p. The animals lost the righting reflex again within a minute and did not regain it for 43 minutes (average S.E. $\pm$9 minutes).

Amphetamine toxicity

Berberine in a dose of 5 mg/kg i.p. did not modify aggregate amphetamine (15 mg/kg i.p.) toxicity, the number of animals surviving in the control as well as berberine treated group being 10%. In contrast, chlorpromazine (1 mg/kg) conferred protection to 70% of the mice.

Berberine also did not significantly alter segregate amphetamine toxicity. The percentage of animals who survived in berberine (5 mg/kg i.p.) treated group was 20% as against 30% survival in group treated with amphetamine (100 mg/kg i.p.).

Berberine on convulsions

Berberine (5 mg/kg i.p.) did not have any action on the convulsions produced by leptazol. The percentage of animals who showed convulsions at dose levels of 20, 50 and 75 mg/kg i.p. of leptazol was 10%, 70% and 90% respectively. The response was unaltered with berberine pretreatment.

Berberine (5 mg/kg i.p.) did not protect mice from supramaximal electroshock convulsions. Thus, all the 10 mice in berberine treated group exhibited convulsions. In contrast, phenobarbitone sodium (20 mg/kg i.p.) offered protection to 50% of the mice.

Pain threshold: (Table 2)

Berberine (5 mg/kg i.p.) did not produce any effect on pain threshold in rats, when

TABLE 2. Effect of berberine (5 mg/kg) on the pain threshold in untreated rats and rats treated with morphine (5 mg/kg) and pentobarbitone (15 mg/kg).

Drug	Dose in mg/kg	Time after administration of drug in minutes	Reaction time in seconds with standard error of mean		p value	% increase (+) or decrease (−) as compared to control
			Before drug	After drug		
Berberine	5	30	3.41±0.33	2.98±0.15	>0.05	− 13%
Morphine	5	45	3.12+0.23	10.8 +1.1	<0.01	+245%
Berberine+ Morphine	5 5	45	3.55±0.1	11 ±1.2	<0.01	+228%
Pentobarbitone	15	10	4.1 +0.27	2.8 +0.14	<0.01	− 32%
		45		3.2 +0.03	<0.01	− 22%
Berberine+ Pentobarbitone	5	10	3.43±0.14	2.66±0.13	<0.01	− 23%
	15	45		3.1 ±0.11	>0.2	− 10%

administered 30 minutes prior to induction of pain. Morphine (5 mg/kg i.p.) increased reaction time almost 3 times. Previous administration of berberine did not modify morphine analgesia significantly.

As expected, pentobarbitone in a sub-hypnotic dose (15 mg/kg i.p.) produced hyperalgesia both at the end of 10 minutes and 45 minutes. Thus, barbiturate treated animals showed a significantly lowered threshold to pain ($p<0.01$). Berberine (5 mg/kg i.p.) pretreatment did not modify barbiturate hyperalgesia.

DISCUSSION

Berberine potentiated the hypnotic action of pentobarbitone and this potentiation was significant at the higher dose levels of the barbiturate. The potentiation of hypnosis could be due to one of the following factors (1). Berberine itself is a depressant of the central nervous system. Hence potentiation of hypnosis is due to an additive effect (2). The liver microsomal enzymes might be inhibited by berberine, thereby preventing the destruction of the barbiturate and increasing its blood level (3). Berberine might facilitate penetration of barbiturate into the brain by altering the blood brain barrier.

Our studies in conscious cats and mice administered berberine by intraperitoneal route, and also our data in conscious cats treated with berberine by intraventricular route has shown that berberine is a sedative. The potentiation of barbiturate hypnosis may therefore be an additive effect.

In the present work, there is no direct evidence of berberine effect on liver microsomal enzyme system. However, there is indirect evidence to suggest that barbiturate potentiation by berberine is a true rather than false potentiation. Jacobsen (8) has stated that addition of a true potentiator increases the percentage of anaesthetized mice, whereas an enzyme inhibitor has no such effect. Also, Fouts and Brodie (9) have put forward the view that a true potentiator will re-induce sleep if administered during awakening. Both criteria are fulfilled by berberine, which therefore appears to be true potentiator.

Aggregate amphetamine toxicity studies suggest that berberine is not a tranquillizer. It is not surprising that berberine, a sedative did not protect mice treated with amphetamine and kept singly in cages. Besides chlorpromazine, even potent hypnotics like pentobarbitone and phenobarbitone are known to be ineffective in altering the LD50 of individual mice (10).

Oreshkov (11) reported remissions of spontaneous pain and tenderness in patients with chronic diseases of bile passages, after treatment with berberine. Our studies indicate that berberine has no analgesic action. In fact, berberine administration has actually reduced the reaction time, though the reduction is not of statistical significance. The reported effectiveness of berberine may therefore be attributed to some other component like its anti-inflammatory action.

The hyperalgesic action of barbiturates in mice has been reported by Neal (12).

Our results show that pentobarbitone in a dose of 15 mg/kg i.p. caused hyperalgesia, 10 and 45 minutes after injection of the barbiturate. It is interesting to note that berberine which potentiated the hypnotic action of pentobarbitone did not influence its hyperalgesia.

Berberine did not protect mice from leptazol and electroshock convulsions and therefore appears to be devoid of anti-convulsant action.

SUMMARY

1. The actions of berberine on the central nervous system are reported in this paper.
2. Berberine produced sedation in conscious cats and mice. It potentiated the pentobarbitone sleeping time.
3. It is devoid of tranquillizing and anticonvulsant property.
4. It has no analgesic action. It did not modify morphine analgesia or barbiturate hyperalgesia.
5. Implications of these findings are discussed.

REFERENCES

1) KAMAT, S.A.: *J. Ass. Phys. Ind.* **15,** 525 (1967)
2) LAHIRI, S.C. AND DUTTA, N.K.: *J. Indian M.A.* **48,** 1 (1967)
3) SUBBAIAH, T.V. AND AMIN, A.H.: *Nature, Lond.* **215,** 527 (1967)
4) DUTTA, N.K. AND IYER, S.N.: *J. Indian M.A.* **50,** 349 (1968)
5) FELDBERG, W. AND SHERWOOD, S.L.: *J. Physiol.* **123,** 148 (1954)
6) JACOBSEN, E.: *Evaluation of Drug Activities, Pharmacometrics,* Edited by LAURENCE, D.R. AND BACHARACH, A.L., Academic Press, London and New York, **1,** 228 (1964)
7) GUPTA, S.K. AND KULKARNI, H.J.: *J. Indian M.A.* **46,** 197 (1966)
8) JACOBSEN, E.: *Evaluation of Drug Activities, Pharmacometrics,* Edited by LAURENCE, D.R. AND BACHARCH, A.L., Academic Press, London and New York, **1,** 232 (1964)
9) FOUTS, J.R. AND BRODIE, B.B.: *J. Pharmac. exp. Ther.* **116,** 480 (1956)
10) LASAGNA L. AND MCCANN, W. P.: *Fedn Proc.* **16,** 315 (1957)
11) ORESCHKOV, T.M.: *Ref. Zh. otd. Vypusk. Farmakol. Toshikol.* **54,** 159, (1965) (As cited in *Biol. Abst,* **47,** 277729, 1966)
12) NEAL, M.J.: *Br. J. Pharmac. Chemother.* **24,** 170 (1965)