EFFECT OF TWO RECENTLY SYNTHESIZED PHENOTHIAZINE DERIVATIVES UPON EXPERIMENTAL CARDIAC ARRHYTHMIAS

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Chlorpromazine has been reported to suppress cardiac arrhythmias produced experimentally (1, 2) and those observed in clinical practice (3). Two new phenothiazine derivatives, namely piperacetazine (2-acetyl-10-[-3-4-(β-hydroxyethyl)piperidinyl]proplyl)-phenothiazine) and benzosulfonate (1-methyl-2-{-(2-methylsulfonyl-dibenzothiazinyl-10)-ethyl-1}-piperidine) have been recently synthesized (4, 5). Hence it is of interest to determine if they possess antiarrhythmic activity.

MATERIAL AND METHODS

Freshly prepared 1% solutions in isotonic saline of benzosulfonate and piperacetazine were used. Cardiac arrhythmias were produced in vagotomized mongrel dogs of both sexes weighing between 8 and 18 kg by the following techniques:

1. Acetylcholine-induced atrial fibrillation

Twelve animals were anaesthetized with intravenous pentobarbital sodium, 30 mg/kg. Atrial fibrillation was produced according to the method of Scherf and Chick (6) which was followed in all essential details as described by Schallek (7). A small pledget of cotton soaked in 5 per cent acetylcholine was placed on the area of sino-atrial node. One minute later, atrial fibrillation was induced by pinching the atrium and its duration noted. Two such controls were taken after which the procedure was repeated a third time. After the atria were fibrillating for a minute, the drug to be tested was injected and reduction in the duration of fibrillation noted.

2. Atrial flutter induced by injury-stimulation

According to the method of Rosenblueth and Garcia Ramos (8), a self-perpetuating type of atrial flutter was produced in 13 pentobarbitalized dogs. A narrow band of atrial tissue connecting the two vena cavae was crushed by a haemostat and flutter was produced by subsequent stimulation of the atria with square waves (duration 1 millisecond, voltage 15 to 20 and frequency 20 per see). The drugs were administered when the flutter had lasted for at least 35 minutes since spontaneous reversion of an arrhythmia of this duration was rare.

3. Aconitine-induced atrial arrhythmia

Atrial arrhythmia was produced by the method of Scherf (9) in 13 dogs which were anaesthetized as above. A cotton pledget soaked in 0.05% solution of aconitine hydro-
chloride was placed on the atrium. Within few minutes, persistent atrial fibrillation or flutter was produced.

In the above procedures (2 and 3), the dosage scheme used was the titration procedure of Winbury and Hemmer (10), that is, 1 mg/kg of the drug was injected every min till reversion to normal sinus rhythm occurred in atrial flutter or end point (1:1 rhythm with the rate below 200 beats/min) was reached in aconitine-induced arrhythmia. Also, in addition to bipolar lead II, electrograms directly from the atria were recorded by a 2-channel Galileo Electrocardiogram.

4. Ventricular arrhythmia following coronary occlusion

In 15 dogs anaesthetised as above, anterior descending branch of the left coronary artery was ligated in 2 stages according to the method of Harris and Kokernot (11). A small incision was made at the left fourth intercostal space and the anterior descending branch of the left coronary artery was exposed. It was dissected free from the connective tissue at a level between 2 and 5 mm distal to the free edge of the left atrial appendage. A double ligature was passed under the artery and cut to provide two ligatures. One was tied snugly but not too tightly around the artery along with a No. 20 hypodermic needle, the needle was withdrawn immediately leaving the artery constricted but not totally occluded. After 30 minutes, the second ligature was tied. The chest wound was closed in layers and natural respiration restored.

The animals were studied in the conscious state 18 to 24 hours after the operation when their electrocardiogram (lead II) indicated heterotopic ventricular beats. The drug, 3 mg/kg, diluted in 10 ml of isotonic saline, was injected intravenously. Same dose was repeated every half an hour, if necessary.

5. Acute toxicity

The 24-hour toxicities of piperacetazine and benzosulfonate were determined following intraperitoneal administration to adult albino mice weighing between 18 and 25 g. LD50 was calculated by the method of Miller and Tainter (12).

RESULTS

1. Acetylcholine-induced atrial fibrillation

Benzosulfonate (1 mg/kg) and piperacetazine (1 mg/kg) brought about a reduction of 84.8±4.4\% (Mean±S.E.) and 63.4±5.7\% (Mean±S.E.) respectively in the duration of fibrillation in 6 experiments with each drug.

2. Atrial flutter

The initial effect of both the drugs was to decrease the rate of atrial flutter. Effect on the ventricular rate was inconsistent. It usually registered a rise at the time atrial rate was decreasing. Increasing the dose resulted in abrupt termination of flutter and restoration of normal sinus rhythm as shown graphically in Fig. 1. The mean doses±S.E. of benzosulfonate (7 dogs) and piperacetazine (6 dogs) causing reversion were 2.2±0.8 mg/kg and 4.4±1.5 mg/kg respectively.
3. Aconitine-induced atrial arrhythmia

The initial effect of both the drugs was to cause invariably a decrease in the rate of fibrillating atria and usually an increase in the ventricular rate resulting in 1:1 rhythm in all the dogs. On increasing the doses, the rate was reduced to 200 beats per minute in 4 out of 6 dogs treated with benzosulfonate, 5.4±1.8 mg/kg, and 5 out of 7 dogs treated with piperacetazine, 6.5±2.4 mg/kg. The results of a typical experiment with each drug are shown graphically in Fig. 1.

4. Ventricular arrhythmia following coronary occlusion

For testing antiarrhythmic activity, only those dogs were selected which exhibited more than 70% ventricular ectopic beats. On the administration of benzosulfonate or piperacetazine, there was at first reduction both in total and ectopic rates. As the action progressed, ventricular ectopic activity was completely eliminated in 3 out of 8 dogs treated with benzosulfonate and 3 out of 7 dogs treated with piperacetazine (Fig. 2). Complete suppressor action lasted for 20 to 40 minutes with the former and 5 to 20 minutes with the latter drug. Thereafter, ectopic activity returned but it was never so marked as before the injection of the drugs.

5. Acute toxicity

Intraperitoneal LD50 of benzosulfonate and piperacetazine were calculated to be 61.2±4.8 mg/kg and 124.4±6.3 mg/kg respectively.
FIG. 2. Effect of benzosulfonate (8 dogs) and piperacetazine (7 dogs) upon ventricular arrhythmia following coronary occlusion. Along the ordinate is ectopic rate (■) and sinus rate (□) per minute. The letter C above each bar shows the control heart rate (sinus and ectopic) before the administration of the drug. The numerals (3, 6 or 9) above the bars, which represent doses in mg/kg, show the heart rates after the administration of the drug.

DISCUSSION

Since the mechanisms involved in the production of cardiac arrhythmias are not clearly understood (13, 14) and since no single animal technique is adequate in investigating the antiarrhythmic activity of a drug (15), a variety of test-procedures have been employed in the present study in order to make an assessment of the spectrum of antiarrhythmic activity of piperacetazine and benzosulfonate. It has been found that both the drugs are effective in causing a significant reduction in the duration of acetylcholine-induced atrial fibrillation, in establishing the end-point in aconitine-induced atrial arrhythmia and in reverting the injury-cum-stimulation-induced atrial flutter to normal sinus rhythm. When these findings are compared with those obtained with quinidine in similar test-procedures in a previous study (16), it is obvious that both these phenothiazines are more potent than quinidine in experimental atrial arrhythmias.

In ventricular ectopic tachycardia following 2-stage ligation of the anterior descending branch of the left coronary artery, both the drugs are effective. Although ventricular ectopic activity is eliminated completely only in some experiments, it is reduced considerably in all cases. It is pertinent to mention here that quinidine and procaineamide, which are antiarrhythmic drugs of established value, fail to cause complete suppression of ventricular arrhythmias accompanying myocardial infarction (17-19). In fact the chief thera-
peutic aim is not to eliminate the ectopic activity completely but only to reduce it sufficiently in order to allow a favourable proportion of sinus impulses to become effective (18); and this is accomplished in all experiments by piperacetazine and benzosulfonate.

Although projection of results from animal experiments to human beings is not always accurate, it may be stated that the persistent hypotensive effect of benzosulfonate (20) and its high acute toxicity (LD50 being 61.2 ± 4.8 mg/kg) preclude the possibility of its being used clinically. On the other hand, piperacetazine shows promise of clinical trials in the treatment of cardiac arrhythmias for the following reasons: (a) Its acute intraperitoneal LD50 in mice is 124.4 ± 6.3 mg/kg which compares favourably with that of quinidine, intraperitoneal LD50 of which was found by Dawes (21) to be 135 mg/kg in the same species. (b) On the basis of its chronic and sub-acute toxicity, the compound has already been introduced in clinical practice as a major tranquilizer (22). (c) It is increasingly recognized that emotional disturbances may precipitate ectopic rhythms in normal hearts as well as in those with structural diseases (23, 24). Further, disorders of rhythm may result in emotional disturbances. This vicious cycle—emotional disturbances causing ectopic rhythms which in turn may lead to emotional disturbances—may be effectively combated by a drug which has both ataractic and quinidine-like properties.

SUMMARY

Two new phenothiazines, benzosulfonate and piperacetazine, have been investigated for their antiarrhythmic activity in the dog. They are effective in reducing the duration of acetylcholine-induced atrial fibrillation, in establishing the end-point in aconitine-induced atrial arrhythmia and in reverting the injury-stimulation-induced atrial flutter to normal sinus rhythm. Also, they cause significant attenuation of ventricular ectopic activity following 2-stage ligation of the anterior descending branch of the left coronary artery. On the basis of their toxicity studies and efficacy, it is suggested that piperacetazine may be useful in the clinic in the treatment of cardiac arrhythmias.

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REFERENCES

1) COURVOISIER, S., FOURNEL, J., DUCROI, R., KOLSKY, M. AND KOETSCHET, P.: Archs int. Pharmacodyln. Thér. 92, 305 (1953)
2) ARORA, R.B. AND MADAN, B.R.: J. Ind. med. Ass. 26, 262 (1956)
3) SATO, J. AND TANABE, Y.: Jap. Circulation J. 26, 216 (1962)
4) KNAPP, L.L., STONE, G.C., HAMBOURGER, W.E. AND DRILL, V.A.: Archs int. Pharmacodyln. Thér. 135, 152 (1962)
5) WEAVER, L.C., MITCHELL, F.E., BURCH, G.R. AND KERLEY, T.L.: Toxic. Appl. Pharmac. 5, 49 (1963)
6) SCHREIF, D. AND CHICK, F.B.: Circulation 3, 764 (1951)
7) SCHALLEK, W.: J. Pharmac. exp. Ther. 105, 291 (1952)
8) Rosenblueth, A. and Garcia Ramos: *Am. Heart J.* 33, 677 (1947)
9) Scherf, D.: *Proc. Soc. exp. Biol. Med.* 64, 233 (1947)
10) Winbury, M.M. and Hemmer, M.L.: *J. Pharmac. exp. Ther.* 113, 402 (1955)
11) Harris, A.S. and Kokernot, R.H.: *Am. J. Physiol.* 163, 505 (1950)
12) Miller, L.C. and Tainter, M.L.: *Proc. Soc. exp. Biol. Med.* 57, 261 (1944)
13) Sekiya, A. and Vaughan Williams, E.: *Br. J. Pharmac. Chemother.* 21, 462 (1963)
14) Szekeres, L. and Papp, J.G.: *Progress in Drug Research* 12, 292 (1968)
15) Bianchi, C., Sanna, G.P. and Turba, C.: *Drug Research* 18, 845 (1968)
16) Madan, B.R. and Pendse, V.K.: *Am. J. Cardiol.* 11, 78 (1963)
17) Harris, A.S., Estandia, A., Ford, T.J., Smith, H.T., Olsen, R.W. and Tillotson, R.F.: *Circulation* 5, 551 (1952)
18) Madan, B.R., Khanna, V.K. and Madan, V.: *Ind. J. Physiol. Pharmac.* 11, 45 (1967)
19) Yeagers, H.C., Scriabine, A. and Bellet, S.: *Archs int. Pharmacodyn. Thér.* 175, 304 (1968)
20) Madan, V.: *Thesis, M.D. Rajasthan University, Jaipur, India* (1970)
21) Dawes, G.S.: *Br. J. Pharmac. Chemother.* 1, 90 (1946)
22) ModeLL, W.: *Clin. Pharmac. Ther.* 10, 749 (1969)
23) Arora, R.B. and Madan, B.R.: *Ind. J. Med. Sci.* 14, 370 (1960)
24) Dreifus, L.S. and Watanabe, Y.: *Am. Heart J.* 70, 291 (1965)