BROMAZINE HYDROCHLORIDE AND COMPLEX COORDINATED REFLEX ACTIVITY

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Bromazine hydrochloride, 2-(4-bromodiphenylmethoxy) ethyldimethylamine hydrochloride (ambodryl), has been made available recently to physicians in Canada. It is related chemically, pharmacologically and therapeutically to diphenhydramine hydrochloride (benadryl hydrochloride). Synthesised by Fleming and Rieveschl its pharmacological properties were first described by Chen, Ensor and Clarke[1], who found that the antihistaminic potency was twice that of diphenhydramine hydrochloride while its acute toxicity was one half that of diphenhydramine hydrochloride. Reports upon its therapeutic use have been reviewed by Boyd and Hicks[2]. The outstanding finding in initial trials on man was that the *p*-bromo-derivative produced fewer side reactions, in particular less drowsiness, than does diphenhydramine. The effect of sub-toxic doses in intact animals has not been reported. In fact, no quantitative measurement of the sedative activity of antihistamines in animals has been reported.

Since depression of complex, co-ordinated, reflex activity is one of the most dangerous side effects of antihistamines in man, producing for example impairment of ability to drive an automobile, it was decided to investigate the effect of bromazine hydrochloride upon a measurement of this cerebral activity in albino rats. The amount of running was measured as a gauge of the degree of complex, coordinated, reflex activity. Running is a complex, co-ordinated, reflex act which is influenced by the urge to run and the physical ability to run. There is no evidence that antihistaminic drugs have any peripheral action upon skeletal muscle or somatic motor nerves in doses that are tolerated by the intact body. These drugs, therefore, cannot affect the physical ability to run. They may or may not affect the urge to run. For the purpose of the argument herein used, the urge to run may be considered as part of the act of running. The urge to run is cortical in origin while the reflexes involved in locomotion involve spinal, midbrain, basal ganglia, vestibular and cerebellar centres[3]. The experiments were designed, therefore, to measure the collective effect of bromazine hydrochloride upon these centres.

Method

The action was determined upon 2 groups of albino rats. The animals were from a stock that has been bred in the animal quarters of the Department of Pharmacology at Queen's University since 1937. They were fed on purina fox chow checkers and water *ad libitum*. The first group consisted of 25 adult albino rats which received the lower doses of the drug by

subcutaneous injection. The group was composed of 15 female and 10 male rats with a body weight of 255 ± 59 g. (mean ± standard deviation). The experiments to be reported upon this group of animals were done during the spring at a room temperature which averaged 24·1 ± 1·8° C., at a mean relative humidity of 42·7 ± 11·4 per cent. and at a mean barometric pressure of 751 ± 1 mm. of mercury. A second group of 35 albino rats, 20 females and 15 males, was used for the administration by mouth doses of bromazine hydrochloride over 10 mg./kg. of body weight. The mean body weight of animals in the second group was 294 ± 66 gm. and the experiments were performed in the summer months at an average room temperature of 26·7 ± 1·3° C., an average relative humidity of 42·2 ± 9·9 per cent., and a mean barometric pressure of 756 ± 3 mm. of mercury.

In order that the effect of bromazine hydrochloride might be compared with that of diphenhydramine hydrochloride, the latter was administered over a corresponding range of doses to 2 further groups of albino rats. The first group was given diphenhydramine hydrochloride by subcutaneous injection over the lower range of doses. This group was composed of 25 albino rats, 15 males and 10 females, with an average body weight of 312 ± 67 g. and the experiments were performed at an average room temperature of 22·8 ± 1·4° C., a mean relative humidity of 35·4 ± 8·7 per cent. and an average barometric pressure of 755 ± 5 mm. of mercury. A second group of 25 albino rats, 15 males and 10 females, of an average body weight of 318 ± 62 g. was given by mouth the larger doses of diphenhydramine hydrochloride under conditions of an average room temperature of 27·3 ± 1·2° C., a mean relative humidity of 49·8 ± 3·7 per cent. saturation and an average barometric pressure of 752 ± 3 mm. of mercury.

For the administration of selected doses of the antihistaminic agents, the animals were divided into sub-groups of 5. One sub-group served as a control while each of the remaining sub-groups received a specified dose of an antihistaminic drug. To prevent possible development of tolerance to the drugs and to allow each animal time to return to a normal state, an interval of one week was allowed to lapse before the albino rats were given again an antihistaminic agent. The experiment was then repeated but the sub-groups were rotated in a cross-over. This procedure was continued at weekly intervals until cross-over was complete and all animals had received in rotation identical treatment.

Measurement of the complex, co-ordinated reflex activity of running was made by means of Wahmann vertically-revolving drums, after a technique described by Shirley,[4] Skinner[5] and Griffith and Farris.[6] The amount of work required to rotate each drum one complete revolution was determined and checked at intervals of approximately one month. The Wahmann drums were carefully cleaned and checked daily with the result that there was little variation in the work per revolution. The mean work required was found to be $6{\cdot}82 \times 10^5 \pm 3{\cdot}32 \times 10^5$ ergs per revolution, in measurements made upon 25 Wahmann drums. The drums were arranged in groups of 5 to receive each sub-group of 5 rats

noted above, with a clock set to time each such group. Each rat was placed in the drum segment of the apparatus, the sliding door between the drum and cage compartments closed, the number on the Veeder counter noted and the timer set at 12 o'clock. After an acclimatisation period, the rat was removed from the drum, the selected dose of the antihistaminic agent administered and the rat returned to the drum. Veeder counter readings were taken at intervals of 1 hour for a period of 2 to 3 hours before, and for 4 to 5 hours after, administration of the drug. The revolutions of the drum and the ergs of work done per rat per hour were then calculated.

TABLE I

THE INCIDENCE OF ULCER FORMATION AT THE SITE OF SUBCUTANEOUS INJECTION OF BROMAZINE HYDROCHLORIDE AND OF DIPHENHYDRAMINE HYDROCHLORIDE IN ALBINO RATS

Dose of antihistamine mg./kg.	Concentration injected per cent.	Incidence of ulcer formation (per cent. of 25 animals)	
		Bromazine hydrochloride	Diphenhydramine hydrochloride
0·5	0·05	0	—
1·0	0·10	—	0
2·5	0·25	0	—
5·0	0·50	12	0
10·0	1·00	—	92
12·5	1·25	68	—
25·0	2·50	80	100

The antihistaminic agents were administered subcutaneously in doses of from 0·5 to 25 mg./kg., each dose per kg. being dissolved aseptically in 1 ml. of sterilised, isotonic saline solution. The control sub-group was given 1 ml./kg. of isotonic saline solution subcutaneously. Both antihistaminic agents produced ulcers at the site of injection and the incidence of ulcer formation has been summarised in Table I. Local induration and ulcer formation at the site of subcutaneous injection of diphenhydramine hydrochloride was reported by Gruhzit and Fisken.[7] It was noted in the study herein reported, that ulcers developed in a majority of animals following subcutaneous injection of a solution containing 1 per cent. or more of either diphenhydramine hydrochloride or bromazine hydrochloride in a volume of 1 ml./kg. of body weight. Two days after injection of such doses, there appeared local swelling and induration, followed by epilation and necrosis of the skin. Between the 7th and 12th days after injection, the necrotic skin fell away, leaving an exposed ulcer which healed between the 10th and 14th days. The presence of the ulcer did not affect the physical activity of the rats. Because of the formation of local ulcers and because the above doses of bromazine hydrochloride and diphenhydramine hydrochloride were found to be without significant effect upon the measured complex, co-ordinated, reflex activity, it was decided to investigate the action of a series of higher doses and these, from 25 mg./kg. of body weight to doses which produced convulsions and death, were given by mouth.

RESULTS

The hourly data upon each group of 25 rats given specified doses of the antihistaminic drugs were averaged. The mean hourly work of the rats when used as controls was calculated. The difference between this mean control erg output and the hourly erg outputs of the rats when given specified doses of the antihistaminic agent was expressed as a percentage of the mean control erg output. These percentages were then plotted in Figure 1.

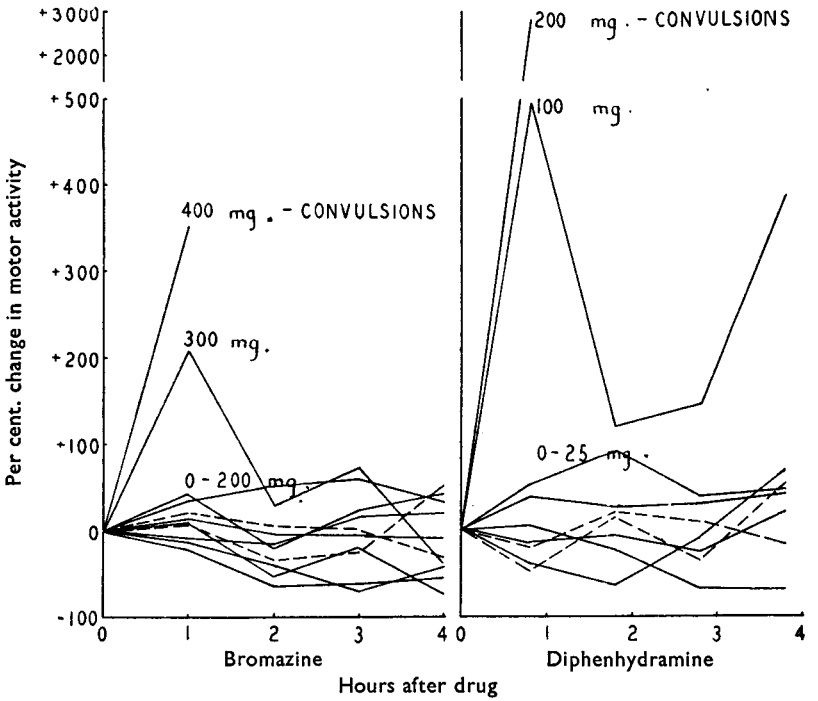

FIG. 1. The percentage effect of increasing doses of bromazine hydrochloride and of diphenhydramine hydrochloride upon a measure of the complex, co-ordinated, reflex motor activity of albino rats. Doses are expressed as mg./kg. of body weight. Controls are shown as interrupted lines.

No dose of bromazine hydrochloride up to and including 200 mg./kg. of body weight had a consistently significant (P less than 0·1) effect upon erg output. Doses of 300 and 400 mg./kg. of body weight significantly increased the erg output. The 400 mg./kg. dose (oral) produced convulsions and killed 80 per cent. of the rats.

Similarly, no dose of diphenhydramine hydrochloride up to and including 25 mg./kg. of body weight had a consistently significant effect upon erg output. Doses of 100 and 200 mg./kg. of body weight significantly augmented erg output, the 200 mg./kg. dose producing convulsions and again a mortality of 80 per cent.

It was not possible by this technique to demonstrate any sedative

activity from lower doses of either bromazine hydrochloride or diphenhydramine hydrochloride. Our results confirm the report of Chen, Ensor and Clarke[1] that bromazine hydrochloride is one half as toxic as is diphenhydramine hydrochloride.

Summary

1. Bromazine hydrochloride was found to have no effect upon a measurement of complex, co-ordinated, reflex activity in albino rats in oral and subcutaneous doses up to 200 mg./kg. of body weight.

2. No effect upon the same measurements occurred following administration of diphenhydramine hydrochloride in doses up to 25 mg./kg. of body weight.

3. Larger doses of both antihistaminic agents augmented the measured reflex activity.

4. Oral doses of 400 mg./kg. of body weight of bromazine hydrochloride and of 200 mg. of diphenhydramine hydrochloride produced convulsions and a mortality of 80 per cent.

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