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EFFECTS OF RAUNESCINE AND ISORAUNESCINE ON BEHAVIOUR AND ON THE 5-HYDROXYTRYPTAMINE AND NORADRENALINE CONTENTS OF BRAIN

BY

M. K. PAASONEN* AND P. B. DEWS

From the Department of Pharmacology, Harvard Medical School, Boston, Massachusetts

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Some behavioural effects of raunescine and isoraunescine on pigeons have been studied; no qualitative difference was detected between their effects and those of reserpine. Isoraunescine is between five and ten times less potent than raunescine, which, in turn, is much less potent than reserpine in producing these effects.

Both raunescine (5 mg./kg.) and isoraunescine (50 mg./kg.) were found to cause a reduction in the concentration of noradrenaline in the brains of rats. Raunescine (5 mg./kg.) also caused a reduction in the concentration of 5-hydroxytryptamine in brain; isoraunescine did not do so in the same dose; higher dose levels were not studied.

In a study of the effects of a number of Rauwolfia alkaloids on the adrenaline and noradrenaline contents of the hearts of rats, it was found that both raunescine and isoraunescine caused a fall in the noradrenaline content (Krayer and Paasonen, unpublished observations).

It has been reported that following administration of reserpine the disappearance and restoration of noradernaline in rabbit brain run parallel to that of 5-hydroxytryptamine. Since it has been suggested that the behavioural effects of Rauwolfia alkaloids may be due to their ability to release from brain 5-hydroxytryptamine (Hess, Shore, and Brodie, 1956) and/or noradrenaline (Holzbauer and Vogt, 1956), the effects of raunescine and isoraunescine both on the 5-hydroxytryptamine and catechol amine content of brain and on behaviour have been studied. For technical reasons, the chemical studies were conducted on rats and the behavioural studies on pigeons.

MATERIALS AND METHODS

Behaviour.—For the experiments on behaviour, pigeons were maintained at a relatively constant level of hunger by keeping them at 80% of the body weight which they attained when fed *ad libitum*. Each day they were exposed to a fixed succession of stimuli in an experimental space. When the behavioural performance had stabilized, observations on the effects of drugs on the performance were started. Details of the experimental procedure have been published (Dews, 1955). Briefly, the pigeons were trained to peck a translucent plastic disk (the key). Each peck broke electrical contacts causing a relay to release. A response is defined as a peck to the key with this consequence. Sometimes, according to a definite schedule, a response led to the presentation of grain to the pigeon for 4 sec. One schedule (designated schedule 14) used was the "multiple schedule" that has been used in previous studies (Dews, 1956). When a red light was present behind the key, grain was presented at the 50th response made (FR); while when a blue light was present, grain was presented at the first response made after an interval of 15 min. had elapsed (FI). The sequence of lights was: R(ed)RRB(lue)RBBRRRRRRRRRRBBR, the changes occurring at food presentation. Five birds were studied on schedule 14. The second schedule (designated schedule 15) was identical with schedule 14 except that the same light was present behind the key, irrespective of whether FR or FI contingencies were in operation. Four birds were studied on schedule 15. Finally, 2 birds were given food at every 50th peck made during a period of 15 min. (FR 50; Dews, 1955). Results were obtained in two forms: as an automatically recorded graph of cumulative number of pecks (ordinate) against time (abscissa) (Figs. 1 and 2) and also as total numbers of pecks made as read from a digital counter.

Brain Chemistry.—For the experiments on brain chemistry, white male rats weighing 160 to 270 g. were used. Whole brains (with the exception of cerebella which were excluded) were removed following decapitation, and were homogenized in saline. The extraction of 5-hydroxytryptamine was done accord-

*Present address: Department of Pharmacology, University of Helsinki, Helsinki, Finland.

ing to the suggestion of Amin, Crawford, and Gaddum (1954) in 95% acetone. Fats were removed by petroleum ether, and the 5-hydroxytryptamine assayed biologically on the heart of *Venus mercenaria*. The conditions and procedure were as described by Paasonen and Vogt (1956) except that real, instead of artificial, sea water was used. For catechol amines, the excised brain was first homogenized with 10% trichloracetic acid and then subjected to the procedure described by v. Euler (1956) with minor changes (Krayer and Paasonen, unpublished observation). The final estimation was done biologically on the blood pressure of the cat and the isolated rectal caecum of the hen (v. Euler, 1948). The cats were given the combination of drugs used by Kärki (1956) before start of the assay.

Reserpine, raunescine, and isoraunescine were dissolved in glacial acetic acid and the solution diluted so that the concentration of acetic acid injected was not in excess of 1%. The drugs were administered intramuscularly to pigeons and intraperitoneally to rats. Control experiments were made using the vehicle alone. In the pigeon experiments, the doses specified are the total doses administered.

RESULTS

Qualitative Considerations

Behavioural Effects.—On schedule 14, the pigeons behaved quite differently according to whether the red or the blue light was on. While the red light was on, the pigeon pecked continuously at a high rate. When the blue light came on, the normal pigeon waited some time before making any response; once it had started to peck there was a period of acceleration followed by sustained pecking at a more or less constant rate until food was presented. Bearing in mind that the slope of the cumulative curve gives the rate of pecking, these various features of the normal performance can be seen in Fig. 1, A. These findings are in full agreement with previously published descriptions (Dews, 1956; Ferster and Skinner, 1957).

The FR performance (the high fixed rate of pecking in the presence of the red light) was much less sensitive to modification by the drugs than was the generally lower and progressively changing rate of pecking characteristic of the FI performance (Fig. 1, B, C, D, E). This same differential sensitivity has been previously described with other drugs (Dews, 1955, 1956). In fact, in none of the experiments illustrated in Fig. 1 did the FR performance differ appreciably from the control; hence the changes in the FI performances cannot have been due to effects of the drugs on the "physical" ability of the birds to peck such as might result, for example, from ataxia.

The effects of raunescine and isoraunescine on FI performance were similar. The normal initial pause succeeded by steady pecking up to the end of the interval was converted to irregular bursts of pecking at a high rate separated by more or less

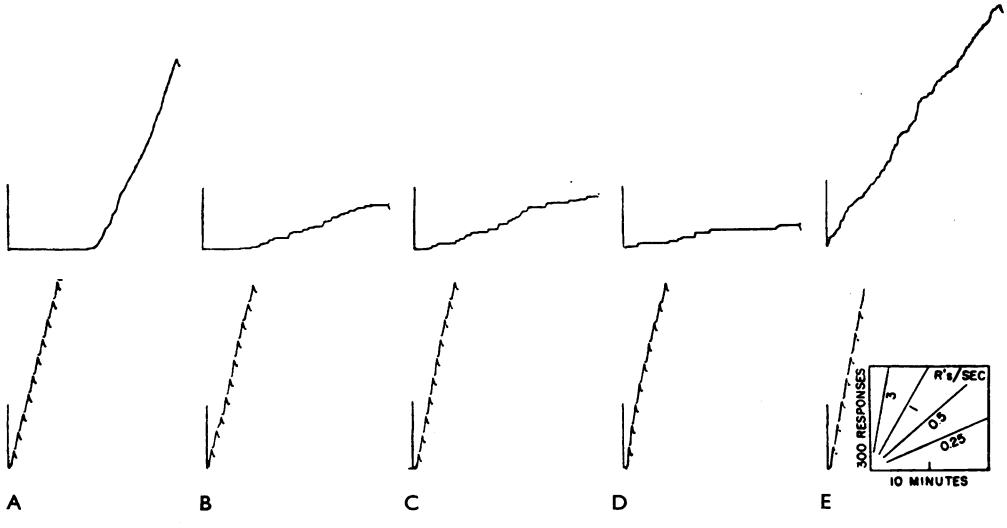

FIG. 1.—The effects of drugs on performance on schedule 14. The upper series of curves shows the cumulative response/time graph (recorded automatically) for the second presentation of the blue light (FI contingency) on a number of days. The lower series shows the sequence of 10 consecutive presentations of the red light (FR contingency) on the same day. The small diagonal hatch marks show the times of food presentation. All records were obtained from the same bird. Times are given to start of the FI contingency. A, control in which no drug was given. B, 30 min. after 1 mg. of raunescine. C, 1 hr. 45 min. after 1 mg. of isoraunescine. D, 3 hr. after 0.05 mg. of reserpine. E, 15 min. after 5·2 mg. of sodium pentobarbitone.

prolonged periods with no pecks (Fig. 1, B, C). The total number of pecks made was greatly reduced. Reserpine had similar effects (Fig. 1, D), but the effects of pentobarbitone were quite different (Fig. 1, E). As previously described, pentobarbitone in appropriate doses caused sustained though irregular pecking throughout the interval (Dews, 1955; Herrnstein and Morse, 1957).

The normal birds on schedule 15 always started pecking at a high rate as soon as the key light appeared. If the food was presented at the fiftieth peck, the 50 pecks were made before a pause occurred. On the occasions when food was presented at a peck only when 15 min. had elapsed, the initial high rate was followed by an abrupt break, and then a succession of pecking rates quite similar to the FI performance of schedule 14 (Fig. 2, F). Purely for the purposes of description, and without prejudice, the following anthropomorphic account can be given. The colour of the key light on schedule 15 gave no information as to whether reward was to occur after 49 pecks or only after 15 min. The pigeon therefore pecked at a high rate until either reward occurred on the fiftieth peck, or until more than 50 pecks had been made without reward, thus indicating that the reward would occur only after the end of the 15 min. period, at which time the FI performance started.

The effects of raunescine and isoraunescine were again to lead to a greatly increased tendency to pause (Fig. 2, G, H), and are indistinguishable from the effects of reserpine (Fig. 2, J). The doses and time intervals for the isoraunescine and reserpine experiments illustrated were such that the "FR" performance was also disturbed (Fig. 2, H, J); the tendency again was for pauses to develop (Dews, 1956).

Quantitative Considerations

The mean number of responses in the FI components of control experiments on schedule 14 was 2,742 and on schedule 15 was 3,674; the coefficients of variation for individual birds averaged 0.22 and 0.18 respectively.

Although effects of both raunescine and isoraunescine were apparent 15 min. after injection (Fig. 1, B, and Table I, pigeons 66 and 165), the maximum effect was not obtained until at least 1.5 hr. had elapsed. A dose of 1 mg. raunescine abolished all responding in experiments on schedule 15 starting 4.25 and 6.75 hr. after injection, and a dose of 0.3 mg. raunescine abolished all responding in an experiment on schedule 14,

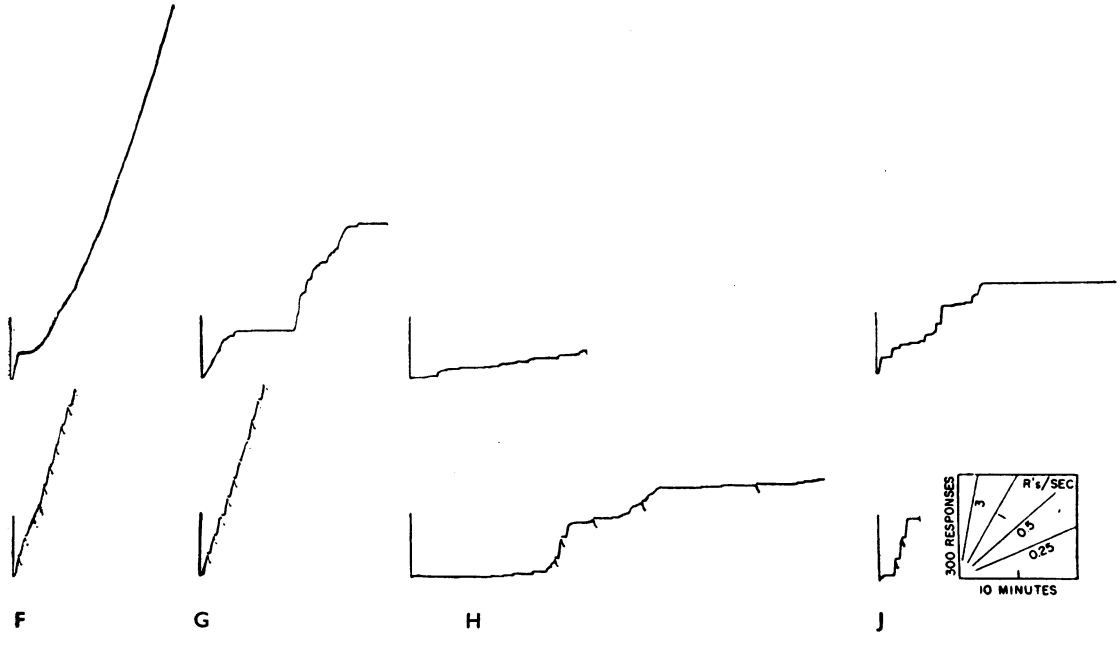

FIG. 2.—The effects of drugs on performance on schedule 15. The general arrangement of this figure is identical with that of Fig. 1, performance under FI contingencies being shown above, and under FR below, although the key light stimuli were constant throughout the experimental session. F, control in which no drug was given. G, 5 hr. 45 min. after 0·1 mg. of raunescine. H, 5 hr. 45 min. after 1·0 mg. of isoraunescine. J, 5 hr. 45 min. after 0·05 mg. of reserpine.

starting 1.5 hr. after injection (Table I). In none of nine experiments, started at various times after injection, did 1 mg. isoraunescine abolish responding. This suggests that raunescine has at least three times the potency of isoraunescine in this regard. On the other hand, in all of four pairs of experiments in which 0.1 mg. of raunescine and 1 mg. of isoraunescine were compared in the same bird at similar times after injection, 1 mg. of isoraunescine showed the greater activity (Table I).

TABLE I

EFFECTS OF RAUNESCINE AND ISORAUNESCINE ON BEHAVIOUR OF PIGEONS

The weight recorded in column 2 is the "running weight" and is about 80% of the weight attained on *ad lib.* feeding. Column 5 records the time between the injection of the drug and the start of the experiment. "Output ratio" is defined as the number of pecks made by the bird while the blue light was on after injection of the drug, divided by the average number made during the blue light on 10 control days. For the description of schedules see text.

Bird (1)	Weight (g.) (2)	Schedule (3)	Dose (mg.) and Drug (4)	Time (hr.) (5)	Output Ratio (6)
66	420	14	1·0 raunescine	0·25	0·10
66	420	14	1·0 isoraunescine	1·5	0·18
1	370	14	0·1 raunescine	3·0	0·74
1	370	14	1·0 isoraunescine	3·0	0·57
165	410	14	1·0 ,,	0·25	0·25
156	440	14	0·3 raunescine	1·5	0
157	500	14	1·0 isoraunescine	3·0	0·10
55	430	15	1·0 raunescine	4·25	0
55	430	15	1·0 isoraunescine	4·25	0·01
62	420	15	0·1 raunescine	5·5	0·84
62	420	15	1·0 isoraunescine	5·5	0·11
166	460	15	1·0 raunescine	6·75	0
166	460	15	1·0 isoraunescine	6·75	0·74
175	400	15	0·1 raunescine	8·0	1·28
175	400	15	1·0 isoraunescine	8·0	0 81
169	420	FR	0·1 raunescine	2·75	0·74
169	420	FR	1·0 isoraunescine	2·75	0·57
172	460	FR	1·0 raunescine	2·75	0·05

Thus, raunescine would appear to have less than 10 times the potency of isoraunescine. Since 0.1 mg. reserpine abolishes all responding for prolonged periods (Dews, 1956), reserpine is, in turn, much more potent than raunescine (probably about 10 times more potent). The results of the three experiments on FR 50 were in agreement with the suggested relative potencies of raunescine and isoraunescine.

Effects on Brain Chemistry.—The concentration of 5-hydroxytryptamine in the brains of rats fell to about half its control value 6 hr. after administration of 5 mg./kg. raunescine. Isoraunescine had no effect on 5-hydroxytryptamine concentration at this dose (Table II). The concentration of noradrenaline was reduced to about one-eighth of its control value by 5 mg./kg. raunescine, and again the same dose of isoraunescine had no effect. However, 50 mg./kg. isoraunescine caused a great reduction in noradrenaline concentration, although probably not as great a fall as that produced by 5 mg./kg. of raunescine. This suggests that

TABLE II

EFFECTS OF RAUNESCINE AND ISORAUNESCINE ON 5-HYDROXYTRYPTAMINE, NORADRENALINE, AND ADRENALINE CONTENTS OF BRAINS OF RATS

Amine content is given in ng./g. wet weight of brain. The time recorded is the interval between injection of the drug and removal of the brain.

Drug	Dose (mg./kg.)	Time (hr.)	Individual Values	Mean and Standard Deviation
			5-Hydroxytryptamine	
Controls ..			345, 348, 368, 385, 386	366±19·6
Raunescine	5	6	139, 160, 168, 230	174
,,	5	16	178, 205, 226, 297	227
Isoraunescine	5	6	350, 352, 360, 412	369
,,	5	16	330, 370, 373, 380	363
			Noradrenaline	
Controls ..			172, 175, 187, 200, 212, 220, 262	204±31·1
Raunescine	5	6	14, 23, 28, 35	25
,,	5	16	18, 46, 53, 70	47
Isoraunescine	5	6	175, 212, 218, 240	211
,,	5	16	220, 230, 232, 265	237
,,	50	6	25, 47, 69, 93	59
			Adrenaline	
Controls ..			10, 17, 20, 26, 31	21±8·1
Raunescine	5	6	11, 14, 13, 18	14
,,	5	16	17, 18, 26, 26	22
Isoraunescine	5	6	7, 15, 16, 18	14
,,	5	16	9, 16, 23, 29	19

raunescine is more than 10 times as potent as isoraunescine in causing this effect. On the other hand, it has been previously shown that 2.5 mg./kg. of reserpine depleted about 80% of brain 5-hydroxytryptamine (Paasonen, unpublished observation), so reserpine is much more potent than raunescine in causing this effect.

The effect of raunescine on the adrenaline content of brain was much less than its effect on noradrenaline.

DISCUSSION

In sufficient dosage, both raunescine and isoraunescine caused behavioural effects which had many similarities to those of reserpine. With the procedure used, the effects of all three drugs were qualitatively indistinguishable. Raunescine and isoraunescine, also like reserpine, caused a reduction in the concentration of both 5-hydroxytryptamine and noradrenaline in the brains of rats. Much larger doses of raunescine and isoraunescine were required to cause the biochemical changes in rats than were required to cause the behavioural effects in pigeons. However, much larger doses of reserpine are required to cause *behavioural* effects in rats than are required in pigeons. While 1 mg./kg. of reserpine was required to abolish lever-pressing behaviour in rats almost completely (Sidman, 1956), about one-fifth of this dose completely abolished all

pecking behaviour in pigeons for 18 hr. (Dews, 1956). The order of potency of the three compounds is reserpine>raunescine>isoraunescine, both behaviourally and biochemically.

The dose of 5 mg./kg. of raunescine caused greater reduction in the brain noradrenaline concentration (to 10% of control) than it did in brain 5-hydroxytryptamine concentration (to 50% of control). Dissociation of noradrenaline and 5-hydroxytryptamine release in this fashion may make it possible to identify which of these two (if either) is related to the behavioural effects of the drugs. The results of these studies are compatible with the postulated indirect effect of the appropriate Rauwolfia alkaloids on the brain, namely that they exert their effect by release of noradrenaline or 5-hydroxytryptamine, and lend some support to this viewpoint.

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