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INCREASE OF 5-HYDROXYTRYPTAMINE IN THE RAT BRAIN BY RAUNESCINE

BY

M. K. PAASONEN AND N. T. KÄRKI

From the Department of Pharmacology, University of Helsinki, Helsinki, Finland

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The Rauwolfia alkaloid raunescine (5 mg./kg., intraperitoneally) increased the concentration of 5-hydroxytryptamine in the brains of rats after iproniazid pre-treatment. This was evident 3 to 4 hr. after raunescine administration. There was no general increase in the noradrenaline content of the brains. In the intestine, raunescine depleted the 5-hydroxytryptamine content by about 50% within 3 to 4 hr. if the animals had been pre-treated with iproniazid. Iproniazid did not increase the content of noradrenaline in the intestine.

When raunescine or reserpine are administered to rats the noradrenaline content of brain and intestine is decreased more rapidly and probably more completely than the 5-hydroxytryptamine content (Paasonen and Dews, 1958; Paasonen and Kärki, 1958; Kärki and Paasonen, 1959). This difference is particularly clear in the intestine. To find out whether the greater depletion of noradrenaline can be explained by destruction of the liberated amine by monoamine oxidase, we treated the rats with iproniazid (Zeller, Barsky, Fouts, Kirchheimer and van Orden, 1952), before administering raunescine. Iproniazid increases the content of 5-hydroxytryptamine and noradrenaline of the brain in various species (Udenfriend, Weissbach and Bogdanski, 1957; Pletscher, 1956a and b, 1957; Paasonen and Giarman, 1958) and also reduces the depletion due to reserpine of these amines in the brain, heart and adrenals (Carlsson, Rosengren, Bertler, and Nilsson, 1957; Pletscher, 1957; Brodie and Shore, 1957).

METHODS

White female rats weighing 120 to 160 g. were used. The animals were not fasted. The experiments were carried out at room temperature (20 to 23°). The whole brain (excluding cerebellum) and about 1 g. of intestine (duodenum and upper jejunum) were taken from each rat. The tissues from two animals were always combined and one half was used for noradrenaline and the other half for 5-hydroxytryptamine determination. The dissection and extraction procedure for noradrenaline was the same as that used in previous work (Kärki and Paasonen, 1959).

For the extraction of 5-hydroxytryptamine the acetone-heptane method (Kärki and Paasonen, 1959) was used. This is essentially similar to the method suggested by Shore and Olin (1958) for catechol amine extraction, with the difference that acetone was used instead of butanol. The tissues were first ground with two volumes 0.01 N-HCl and sand, then extracted with 90% (v/v, final concentration) acetone, containing 4 g. NaCl. After shaking for 1 hr. the aqueous phase was separated by the addition of heptane. This phase still contained some acetone which was evaporated *in vacuo* at 35°. With this method, the recoveries of added 5-hydroxytryptamine have been 80% or more. No correction was made for the loss of 5-hydroxytryptamine due to the extraction method.

The blood was collected after decapitation in tubes containing heparin and extracted with 95% acetone (Amin, Crawford, and Gaddum, 1954).

The amines were assayed biologically by using the cat blood pressure method for noradrenaline (Kärki, 1956) and the rat stomach method of Vane (1957) for 5-hydroxytryptamine.

Iproniazid phosphate (Hoffmann-La Roche) was injected subcutaneously in a dose of 100 mg./kg. of the base. In each experiment, all the animals were injected with iproniazid at the same time. Raunescine was administered from 14 to 17 hr. after iproniazid and the animals were then kept for various periods—up to 6 hr.—before they were killed. Control animals treated with iproniazid alone were kept alive on the average for the same period of time. The survival times after iproniazid of the control and experimental animals differed by not more than 2 hr. Since the brain 5-hydroxytryptamine and noradrenaline remain elevated after iproniazid for at least 48 hr. (Spector, Prockop, Shore, and Brodie, 1957; Paasonen and Giarman, 1958) it seemed unlikely that differences of 2 hr. in survival time after iproniazid would have been significant.

The raunescine (S. B. Penick & Co., N.Y.) was dissolved in glacial acetic acid and the solution diluted so that the final concentration of acetic acid

TABLE I

EFFECTS OF IPRONIAZID AND RAUNESCINE ON 5-HYDROXYTRYPTAMINE AND NORADRENALINE CONTENT OF THE BRAIN AND SMALL INTESTINE OF THE RAT

All values are ng./g. wet weight. Each column contains results obtained on the same day. Iproniazid (100 mg./kg. subcutaneously) was injected about 18 hr. before killing the animals. The time interval is that between the injection of raunescine (5 mg./kg. intraperitoneally) and the removal of the tissues.

Drug	Time (hr.)	5-Hydroxytryptamine	Mean	Noradrenaline	Mean
		Brain			
None ..		248 279 213 420 340 457 332 316	326	140 140 230 340 169 326 323 200	234
Iproniazid ..		526 564 315 1,320 1,050 1,130 1,060 790 588	816	274 273 543 615 315 410 540 475	443
,,+raunescine	1	857 840 1,040 550 670	791	385 245 600 436	417
,, ,,	2	1,340 790 740	957	355	355
,, ,,	3	2,250 1,300 1,200 980 870	1,103	490 208 370 405	344
,, ,,	4	778 770 795 980		258 263 303 495	
,, ,,	6	990 569 842	800	181 625 342	383
		Intestine			
None ..		2,130 2,250 5,000 3,200 4,530 6,200 4,320	3,950	243 250 180 210 255 410 270	260
Iproniazid ..		4,350 6,600 4,500 4,620 7,060 7,330 6,110	5,800	460 280 155 235 161 217 195	232
,,+raunescine	1	4,940 5,520 4,740	5,070	270 113 80	154
,, ,,	2	2,680	2,680	128	128
,, ,,	3	2,810 5,000 3,260	2,970	93 72 82	62
,, ,,	4	2,270 1,880 1,050 4,500		85 48 39 41	
,, ,,	6	7,250 4,630	5,940	57 37	47

injected was not in excess of 2%. The dose of raunescine was 5 mg./kg. and it was given intraperitoneally in a solution containing 2.5 mg./ml. 5-Hydroxytryptamine was used as creatinine sulphate (Abbott Lab., Chicago, Ill.) and noradrenaline as bitartrate (Bayer Products, London). In each case the dose refers to the base.

RESULTS

The detailed results on brain and intestine are presented in Table I. For unknown reasons the scatter in the control groups was rather great, but values obtained at the same time and under strictly identical conditions were comparable. In three pairs of control rats, taken at the same time, the highest value was in no case more than 10% higher than the lowest. Consequently results obtained on the same day are shown in the same columns. The mean % changes are shown in Figs. 1 and 2 and their statistical significance is indicated in the text.

Brain

5-Hydroxytryptamine. — Iproniazid treatment increased the 5-hydroxytryptamine content in brain ($P<0.001$), but there was some indication that raunescine, when allowed to act for 1 hr., lowered the 5-hydroxytryptamine content below that of the iproniazid controls. After 3 to 4 hr., however, there is a mean increase of 44%, which was statistically significant as compared with the control ($P<0.05$). The values obtained 6 hr. after injection of raunescine indicate that the content of 5-hydroxytryptamine had fallen to the pre-injection level or below (Fig. 1).

These findings are in marked contrast to those obtained with raunescine without iproniazid pretreatment when a dose of 5 mg./kg. of raunescine lowered the 5-hydroxytryptamine content by some 40% within 4 hr. (Kärki and Paasonen, 1959).

Noradrenaline.—Iproniazid increased the amount of noradrenaline in brain ($P<0.01$). After raunescine there was a decrease of about 20% of the amine as compared with controls treated with iproniazid alone, but the difference was not statistically significant.

Intestine

5-Hydroxytryptamine.—In the intestine the % increase of the 5-hydroxytryptamine content by iproniazid was not as great as that in brain, but it was statistically significant ($P<0.05$). After raunescine, the 5-hydroxytryptamine content was decreased compared with controls treated with iproniazid alone. The reduction was statistically significant ($P<0.01$) 3 to 4 hr. after the administration of raunescine.

Noradrenaline.—Iproniazid produced no significant change in the noradrenaline content of the intestine, but raunescine given after iproniazid produced a considerable depletion of noradrenaline ($P<0.01$).

Blood

5-Hydroxytryptamine.—In three experiments the blood from normal rats, rats treated with iproniazid and those treated with iproniazid plus

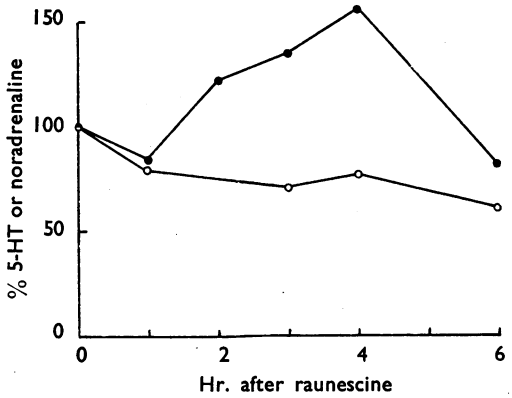

FIG. 1.—Mean % changes (derived from Table I) in 5-hydroxytryptamine (5-HT), ●——●, and noradrenaline, O——O, content of brain caused by raunescine in rats pre-treated with iproniazid.

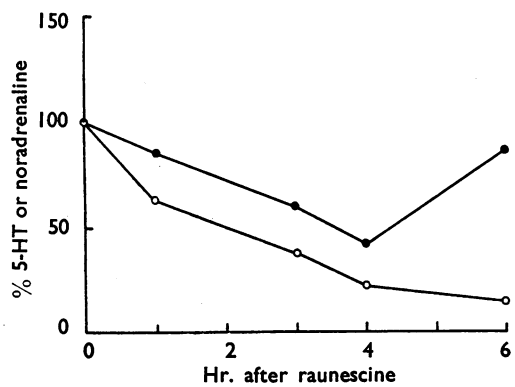

FIG. 2.—Mean % changes in 5-hydroxytryptamine (5-HT), ●——●, and noradrenaline, O——O, content of intestine caused by raunescine in rats pre-treated with iproniazid.

raunescine (1, 3 and 6 hr.) was analysed. The following is a typical example. Content of 5-hydroxytryptamine (ng./ml.) in untreated control, 210; after iproniazid, 504; after iproniazid plus raunescine, at 1 hr. 240, at 3 hr. 130, and at 6 hr. 93.

Symptoms Observed

Iproniazid alone increased motor activity in rats. This was accompanied by slight piloerection, exophthalmos and mydriasis. Raunescine increased all these symptoms. The effect was clear within 5 to 10 min. and lasted for the whole observation time. However, there was usually some indication that the excitement and other symptoms were decreased 5 to 6 hr. after the injection of raunescine.

DISCUSSION

These experiments show that 3 to 4 hr. after administration of raunescine in iproniazid pre-treated rats there was a significant increase in brain 5-hydroxytryptamine. A possible explanation of this increase is that 5-hydroxytryptamine, which is located in intracellular granules (Blaschko, 1957) is normally destroyed by monoamine oxidase when the former is released from its binding sites. Iproniazid prevented this destruction and we may assume that the cells were trying to replace the "vacant" space in the granules by synthesizing more 5-hydroxytryptamine, thus increasing the total content of this amine.

Another possible explanation of the increase in 5-hydroxytryptamine is that the brain may be able to pick up some of the 5-hydroxytryptamine liberated from other tissues. The blood findings, however, showing a consistent fall in iproniazid-raunescine animals, did not support this explanation. On the other hand, the platelets become unable to bind 5-hydroxytryptamine (Shore, Pletscher, Tomich, Kunzman, and Brodie, 1956), which may be of significance. We are establishing whether the 5-hydroxytryptamine injected into rats will penetrate the brain tissue more easily after raunescine.

In the intestine, there was no evidence of an increase of 5-hydroxytryptamine after raunescine in the iproniazid-treated animals. It is interesting and relevant to note that raunescine was able to release about half the intestinal 5-hydroxytryptamine after iproniazid, although it is almost inactive in this respect in animals which have not been pre-treated with iproniazid (Kärki and Paasonen, 1959).

Apart from a few estimations, there was no indication of elevated levels of noradrenaline after raunescine in iproniazid-treated rats. This agrees with the findings of Pletscher (1957) that iproniazid increases the content of 5-hydroxytryptamine in rat brain more than the content of noradrenaline and suggests that the rôle of amine oxidase may be more significant in the katabolism of 5-hydroxytryptamine than it is in that of noradrenaline (see Corne and Graham, 1957).

We may assume that the mechanism responsible for the elevation of brain 5-hydroxytryptamine after raunescine in rats pre-treated with iproniazid also works in animals not treated with iproniazid. The increase of 5-hydroxytryptamine may be masked by the simultaneous depletion of the amine due to the rauwolfia alkaloid. It has previously been shown that noradrenaline is

released more completely than 5-hydroxytryptamine from the brains of rats after reserpine and raunescine (Paasonen and Dews, 1958; Kärki and Paasonen, 1959). The brain is thus differentially depleted of the two amines in both normal and iproniazid-treated rats.

When raunescine is administered alone, it produces sedation and lowers the content of noradrenaline in the brain even when there is no demonstrable change in 5-hydroxytryptamine in this organ (Kärki and Paasonen, 1959). On the other hand, when raunescine is administered in the presence of iproniazid it stimulates, as shown both in the present experiments and in those of Brodie and Shore (1957) on rabbits, and increases 5-hydroxytryptamine content. We do not know whether this increase represents the "free" or "bound" amine as defined by Brodie (1958). It is conceivable that in each case the pharmacological effects are produced by free 5-hydroxytryptamine (that which has been released from its binding sites into the cytoplasm). It would then follow that low concentrations of free 5-hydroxytryptamine are depressant while high concentrations are stimulant as suggested by Brodie (1958). There is, at present, no direct evidence for this theory.

This study leaves unanswered the question whether or not noradrenaline and/or 5-hydroxytryptamine is the most important mediator in the action of the rauwolfia alkaloids.

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