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Biochemical Pharmacology, Vol. 19, pp. 2169–2172. Pergamon Press. 1970. Printed in Great Britain

p-chlorophenylalanine depletion of gastrointestinal 5-hydroxytryptamine*

(*Received* 29 *July* 1969; *accepted* 10 *December* 1969)

5-HYDROXYTRYPTAMINE (serotonin, 5-HT) is widely distributed throughout the mammalian organism. The organ system which has the highest concentration of this amine is the gastrointestinal tract. The distribution of 5-HT in the gastrointestinal tract varies greatly from one portion to another. The distribution of 5-HT in the rat gastrointestinal tract has recently been carefully studied.[1] With the

* A preliminary report presented at Western Pharmacology Society, January 24–26, 1969.

existence of relatively large concentrations of 5-HT in the gastrointestinal tract, it might be expected that the rate of anabolism for tryptophan hydroxylation and decarboxylation would be high or the rate of catabolism would be low. There is a fairly rapid decarboxylation system in the gastrointestinal tract,[2] but the hydroxylation of tryptophan as measured *in vitro* does not appear to be as great as in other organs containing 5-HT.[3]

The role of 5-HT in the intestine is uncertain, but it does have some effect on tone and motility and may act as a ganglionic neurotransmitter.[4] The effect of 5-HT application and release of 5-HT via agents such as reserpine have been investigated.[2] In the rat, complete or even near complete depletion of 5-HT is difficult to achieve and synthesis of 5-HT from tryptophan continues unaltered after reserpine. With the discovery of the tryptophan hydroxylase inhibitor, *p*-chlorophenylalanine (PCPA)[5] a potential tool became available to cause a depletion of 5-HT from the gastrointestinal tract. Colon has been reported to have 5-HT content lowered.[5] The purpose of this investigation is to examine PCPA as a depletor of 5-HT in various portions of the gastrointestinal tract.

Male Sprague–Dawley rats weighing 250–350 g were used. PCPA (200 mg/kg) suspended in saline (0·9%) and Tween "80", 1%, were injected intraperitoneally daily. Control animals were injected with the same volume of saline and Tween. The animals were injected and sacrificed in midmorning (10:00 to 11:00 a.m.) to avoid diurnal variation.

The animals were sacrificed by crushing the spinal column in the neck and exsanguinated. The tissues were quickly removed and chilled. The portions of gastrointestinal tract were cut open and gently washed until they were clean of fecal and food material. The tissues were blotted dry, weighed and homogenized in 0·1 N HCl.

The rat stomach was divided into corpus and antrum as described by Gregg.[6] The fore stomach was not used because initially the author found minimal amounts of 5-HT to be present at this location. The first 9 cm of the small intestine was used as duodenum, the last 22 cm before the caecum was used as ileum, and the entire intestine between these two portions was used as jejunum. The caecum and colon were individually assayed. Photofluorometric assay of 5-HT was performed as described by Udenfriend *et al.*[7]

Tissue blanks for intestine are difficult to obtain as reserpine does not cause 5-HT depletion of gut as it does brain. The method for tissue blanks with PCPA and control animals was as follows: Samples of tissue were prepared as 10% homogenates at 0° with 0·067 M phosphate buffer, pH 7·4. The antrum of the stomach was prepared as a 2·5% homogenate because of the limited amount of tissue available. The 2-ml homogenate was then incubated 1½ hr with 1 ml of a 33⅓% homogenate of control liver as a source of monoamine oxidase and 1 ml of buffer. Because of different concentration of tissue, zero time incubations were done for comparative purposes. At the end of the incubation period, the tissues were photofluorometrically assayed for 5-HT. To verify this method for determining tissue blanks, 1 μg of 5-HT was added to liver controls and to liver plus intestine samples. All of the added 5-HT was destroyed by incubation in the above manner. The possibility exists that all 5-HT in the homogenate is not available; attempts by excessive homogenation under ice and repeated freezing and thawing did not alter the blank readings.

Close record was kept during the experiment to see if PCPA treatment had any significant effect on the weight of the animals or on individual tissue weights. No significant change was noted.

TABLE 1. AMOUNT OF 5-HYDROXYTRYPTAMINE (5-HT) IN μg per gram TISSUE WITH STANDARD ERROR (S.E.) FOR TWENTY CONTROL RATS

Control 5-hydroxytryptamine levels		
Rat tissue	5-HT μg/g	± S. E.
Brain	0·55	0·07
Stomach		
Corpus	1·83	0·09
Antrum	9·88	0·31
Duodenum	5·12	0·20
Jejunum	2·80	0·14
Ileum	3·02	0·20
Caecum	7·07	0·24
Colon	5·24	0·14

Although depletion of 5-HT in the brain was not an objective in this study, assays were performed as a positive control since numerous investigators have looked at brain 5-HT levels. Table 1 gives values for the amount of 5-HT in μg per gram wet weight of tissue found in twenty control animals The rest of the data will be presented as a percentage of this control value. Figure 1 shows per cent changes of 5-HT in the brain and the gastrointestinal tract. The brain is rapidly depleted reaching a value of 10 per cent of the control level within 3–4 days of PCPA treatment. The antrum of the stomach is also depleted but only to a value of about 30 per cent, reaching this value within 2–3 days of treatment. The corpus on the other hand was not depleted of 5-HT except on the fourth day.

The small intestine is slowly but incompletely depleted of 5-HT by pretreatment with PCPA. After 6 days all portions of the small intestine are decreased to 40–50 per cent of normal and it is apparent

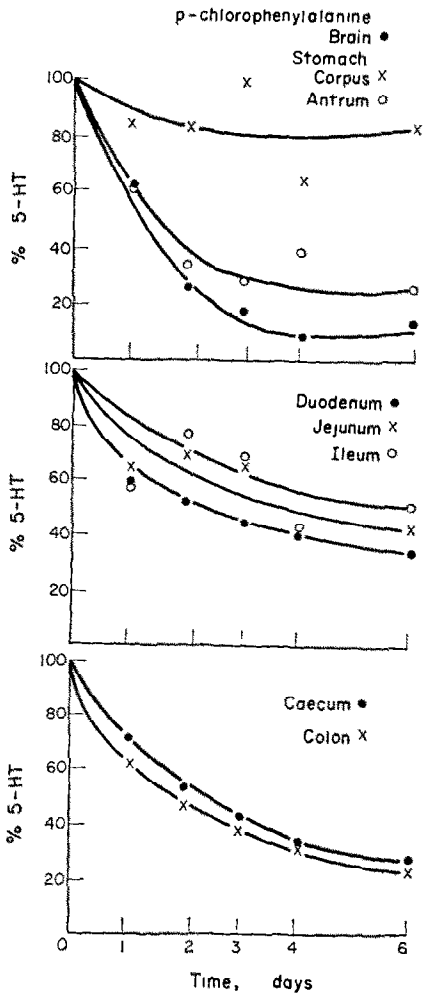

FIG. 1. The effect of *p*-chlorophenylalanine (PCPA) on 5-hydroxytryptamine (5-HT) in the gastrointestinal tract and brain. Control No. 20, and all other points are from 6 separate experiments Significance as determined by Student *t*-test is as follows: brain, antrum of stomach, caecum and colon $P = > 0{\cdot}001$ each day; corpus not significant on day 1, 2, 3, and 6, $P = < 0{\cdot}01$ on day 4; duodenum $P = < 0{\cdot}01$ on day 3, all others $P = < 0{\cdot}001$; P values for the following days for jejunum are $1 = < 0{\cdot}01$, $2 = < 0{\cdot}05$, $3 = < 0{\cdot}01$, 4 and $5 = < 0{\cdot}001$; for the ileum $1 = < 0{\cdot}01$, 2 = not significant, $3 = < 0{\cdot}05$, 4 and $6 = < 0{\cdot}001$.

that there is still a small but steady negative slope for 5-HT decrease. The caecum and colon are depleted of 5-HT at a fairly even rate to 25 per cent of control levels at 6 days for both tissues. It evident here again that a slight negative slope is occurring between the fourth and sixth day.

In situ *p*-chlorophenylalanine (PCPA) decreases the level of 5-hydroxytryptamine (5-HT) in the gastrointestinal tract in a manner similar to that of the brain. However, the degree of depletion and the rate of decrease of 5-HT in the gastrointestinal tract is different than that occurring in the brain In no portion of the intestine is the 5-HT decreased to as great an extent as that in the brain.

It is interesting that the slope of depletion of the antrum of the stomach and the brain is very similar. The rest of the gastrointestinal tract has a much shallower slope. The brain has the least amount of 5-HT per gram of tissue and the antrum the largest amount of 5-HT of the tissues studied here. This large difference in quantitative amounts, coincidental with similar depletion rates of 5-HT by PCPA, might indicate that the anabolism or catabolism of 5-HT is similar in these tissues.

The corpus of the stomach is perhaps the most interesting of the tissues studied, in that almost no depletion of 5-HT occurs even though it has a relatively high concentration of 5-HT. No explanation for this lack of depletion of 5-HT is evident from these experiments.

Acknowledgements—The author thanks Miss Catherine Busche and Miss Candace Jenkins for their help.

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Biochemical Pharmacology, Vol. 19, pp. 2172–2176. Pergamon Press. 1970. Printed in Great Britain

Reversal of the growth inhibitory effects of 6-methylthiopurine ribonucleoside

(*Received* 26 *September* 1969; *accepted* 10 *December* 1969)

MeMP*-RIBONUCLEOSIDE, an agent of interest because of its antitumor activity in experimental systems,[1] is metabolized in mammalian cells to the monophosphate derivative, but not to the di- or triphosphates.[1,2] A metabolite of MeMP-ribonucleoside strongly inhibits an early step of purine

* Abbreviations: MP, 6-mercaptopurine; MeMP, 6-methylthiopurine; AIC, 4-amino-5-imidazole-carboxamide; PRPP, 5-phosphoribosyl-1-pyrophosphate.