METABOLITES OF PIPERIDINE IN RAT URINE

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Abstracts—Piperidine is one of pharmacologically active biogenic amines. While two pathways for piperidine production have been reported, little is known about metabolism of the compound. In the present study, piperidine and its hydroxylated, conjugated and unknown metabolites were detected in rat urine by radiochromatographic analysis. Using GC-MS technique, it was confirmed that 3-hydroxypiperidine and 4-hydroxypiperidine are major metabolites of piperidine of either exogenous or endogenous origin. The findings substantiate the existence of a mechanism which inactivates piperidine in the living body, since both metabolites lack the potent pharmacological activities as those induced by piperidine.

Piperidine (Pip) is a normal constituent of brain (1, 2, 3, 4, 5, 6, 7), skin (8, 9) and urine (3, 10) of mammals, and of the brain (11), cerebrospinal fluid (12), and urine (3, 10, 13) of humans, and of cerebral ganglia of molluscs (14). Pip shows potent nicotine-like actions on the peripheral (15, 16, 17) and central nervous systems (CNS) (18, 19). Pip acts on the autonomic ganglia, neuromuscular junctions and chemoreceptors, i.e., it elicits stimulation in small doses and depression in large doses. In the CNS, it produces synaptic stimulation followed by depression (19), exerts tranquilizing effects on schizophrenic patients (20) and counteracts experimentally induced aggressiveness in mice and rats (19). The findings obtained from chemical stimulation of the brain by Pip suggest that the amine itself affects neural mechanisms governing regulation of emotional behaviour, sleeping and extrapyramidal function (21, 22).

Pip can be produced in vitro (23) as well as in vivo (24, 25) from pipecolic acid, which is an intermediate of lysine metabolism and found in serum (26), urine (27) and the brain (28) of mammals. On the other hand, another possible pathway of Pip production from cadaverine has been reported in invertebrates (29).

A study using tritiated Pip on the subcellular distribution of the amine shows that 3H-Pip is mostly localized in the nerve ending particles after subfractionation of the rat brain (30). It is also suggested that there are uptake and storage mechanisms for Pip in nerve endings (31).

Thus, Pip plays an important role in neural mechanism of the brain, and the present authors (22) and others (32) have assigned a modulator role to Pip in the CNS. On the

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other hand, there is little information on the metabolism of Pip. In the present study, metabolites of Pip in the rat urine were studied in order an attempt to elucidate the mechanism of inactivation of the amine in living body.

MATERIALS AND METHODS

Detection of metabolites using 3H-labelled piperidine

3H-Pip hydrochloride (specific activity, 300 \( \mu \)Ci/mg) was prepared by New England Nuclear Corp. according to the method of Wilzbach (33). Unstable tritium was removed by repeated crystallization to a constant specific activity and pure crystals of the salt were used. Glusulase used for the present experiment was prepared by Endo Laboratories Inc. and contains 100,000 units of \( \beta \)-glucuronidase and 50,000 units of aryl-5-sulfatase per ml.

Twenty-four male Wistar rats, weighing 180±20 g, were divided in two groups. 3H-Pip HC1 (50 \( \mu \)Ci/animal) was given i.p. to each of 12 rats of one group (Pip group). Rats of the other group (control group) were given the same volume of saline. Total urine of each group was collected over a 72 hour period under toluene layer (5 ml) with 1N HC1 (10 ml) in a flask. Each pooled urine was extracted by a mixture of cyclohexane and benzene (1:1). After evaporation, the residue was dissolved in a small volume of ethanol, and the solution was spread in a narrow band on a chromatograph paper (Toyo-Roshi, No. 51, 3 x 57 cm). Analysis by paper chromatography was performed as follows: developing solvent, \( n \)-butanol : acetic acid : water = 4:1:2, ascending method, for 24 hours. After drying, the chromatograms were scanned on a radiochromatography scanner (RCS, Aloka-type JPC 102).

In order to detect the presence of conjugated Pip, a part of the urine described above was adjusted to pH 6.5 with 2N NaOH. Both phosphate and sulfate ions were removed from the urine by adding an excess of saturated BaCl\(_2\) solution and by centrifuging the precipitate. The supernatant was incubated with 0.1 ml of \( \beta \)-glusulase for 24 hours at 37\(^\circ\)C (34), extracted and subsequently treated by the same procedure as described above.

Prior to this experiment, Rf-values of authentic Pip and its putative metabolites such as 3-hydroxypiperidine (3-OH-Pip), 4-hydroxypiperidine (4-OH-Pip), N-acetyl-piperidine, N-methylpiperidine and piperidone-2 on paper chromatogram were determined by using ninhydrin and Dragendorff's reagents.

Identification and quantitation of metabolites

Four groups each including nine rats were used. Pip HCl (1.0 mg/animal) was given i.p. to each rat of two groups (pip groups) and the same volume of saline to each rat of the other two groups (control groups). Urine from each group was collected over a 72 hour period. The pooled urine was treated with the same volume of 5\% trichloracetic acid solution (TCA). After centrifugation, the supernatant was adjusted to pH 12 with 2N NaOH and the amines in the solution were converted to dinitrophenyl (DNP) derivatives according to the procedures previously described (23, 24, 25).

The DNP derivatives produced were extracted with 50 ml of cyclohexane and then with the same volume of ethyl acetate. After evaporation, the residue was dissolved in a
small volume of mixed solvents of benzene, cyclohexane and ethyl acetate (1:1:1). The extract was spread in a narrow band on a thin-layer chromatograph (TLC, 20 x 20 cm glass plate, 100 μm thick, Wakogel B-5) and developed with chloroform, using the ascending method, for 30 min. Authentic samples of DNP-Pip, DNP-3-OH-Pip and DNP-4-OH-Pip were also run parallel with the samples described above. Each spot, corresponding to each of Rfs of authentic DNP-derivatives, was scraped off and eluted with a mixture of benzene and ethyl acetate (1:1). After evaporation in vacuo, the residue was dissolved in a fixed volume of ethanol. A part of the solution (0.4-2.0 μl) was analyzed with a gas chromatograph-mass spectrometer.

Gas chromatography-mass spectrometry (GC-MS): Following the gas chromatographic separation, mass spectrometry was utilized for identification, and selected ion monitoring technique was also adopted for quantitation of Pip metabolites.

Gas chromatographic separation was performed by using a 2 mm x 1 m glass column packed with 1.5% OV-17 on Shimalite W (80 to 100 mesh). Chromatographic conditions: column temp., 200°C; injection port temp., 300°C; ion source temp., 230°C; He flow rate, 40 ml/min. Mass spectral studies were carried out with a double focussing mass spectrometer (JEOL GC-MS D-100). Mass spectral conditions: electron energy, 25-75 eV; trap current, 300 μA; accelerating voltage, 3 KV; ion multiple voltage, 1.2-1.75 KV (35, 36). The quantitation of hydroxylated Pip was performed by selected ion monitoring (SIM), and the ion employed was m/e 250. Quantities were read from a calibration curve which had been prepared by plotting intensities of the fragment ions of authentic DNP-3-OH-Pip and DNP-4-OH-Pip against concentration of both compounds.

RESULTS

Detection of metabolites

As shown in the upper record of Fig. 1, five peaks appeared on the radiochromatogram of samples obtained from rat urine. By comparing the Rf-value of each peak with that of corresponding authentic compounds, the peaks were identified as follows. The highest peak (M₄) was identical with Pip itself, and the peak M₂ with 3-OH-Pip or/and 4-OH-Pip.  

![Radiochromatograms](image)

**Fig. 1.** Radiochromatograms of the sample obtained from rat urine. Upper: Before treatment with β-glusulase. M₁ represents a peak for ³H-Pip itself and the remaining four peaks represent the metabolites of ³H-Pip. Lower: After treatment with β-glusulase. Both M₂ and M₃ disappeared.
However, the remaining three peaks such as M1, M3, and M5 were identical with none of authentic compounds such as N-acetylpiperidine, N-methylpiperidine or piperidone-2. After treatment with β-glusulase, the peaks M3 and M5 disappeared as shown in the lower record of Fig. 1.

Identification and quantitation of metabolites

Authentic DNP-3-OH-Pip and DNP-4-OH-Pip were applied to GC-MS and peaks appeared on chromatograms of total ion monitor (TIM) were analyzed by mass spectrometry. Mass spectra of both authentic amines are shown in Fig. 2. Major fragment ions of both amines were as follows. 1) DNP-3-OH-Pip : a parent ion peak at m/e 267, a base ion peak at m/e 232 and another intensive fragment ion peak at m/e 250. 2) DNP-4-OH-Pip : a parent ion peak at m/e 267, a base ion peak at m/e 232 and another intensive fragment ion peaks at m/e 250 and 186.

The sample obtained from the urine of the Pip group was then applied to GC-MS. Two peaks having retention times equal to those of authentic DNP-3-OH-Pip and DNP-4-OH-Pip (9.9 and 12.1 minutes, respectively) appeared on the TIM chromatogram. Mass

Fig. 2. Mass spectra of authentic DNP-3-hydroxypiperidine (upper) and DNP-4-hydroxypiperidine (lower). The pattern of mass spectrum of the sample obtained from the Pip group or the control group was identical with that of authentic DNP-3-OH-Pip (upper). In other words, 3-OH-Pip was present not only in the urine of the Pip group but also in that of the control group. The same holds true in the case of DNP-4-OH-Pip (lower).

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<th>Table 1. Quantities of hydroxylated piperidines in rat urine</th>
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spectra of the peaks were quite identical with those of DNP-3-OH-Pip and DNP-4-OH-Pip (Fig. 2). The sample from the control group also showed the same pattern of spectra as those of Pip group, that is, both 3-OH-Pip and 4-OH-Pip were found in the urine of the control group.

The quantities of hydroxylated Pip measured are shown in Table 1. The quantities of 3-OH-Pip and 4-OH-Pip in the Pip group were 2.26 and 3.12 times larger than those of corresponding compounds in the control group, respectively.

DISCUSSION

Pip is one of biogenic amines possessing potent pharmacological and physiological actions (15–22). The present study was undertaken to determine the metabolic fate of Pip in the living body.

Five peaks (M1-M5) appeared on the radiochromatogram of the sample obtained from urine of rats administered \(^{3}\)H-Pip. Two were identified with Pip (M4) and hydroxylated piperidine (M3), respectively. GC-MS analysis revealed that the major metabolite (M2) consisted of two components identical with 3-OH-Pip and 4-OH-Pip. Another two (M3 and M4) were assumed to be conjugated compounds of Pip or those of metabolites because they disappeared after treatment with \(\beta\)-glusulase. However, further elucidation of the compounds and identification of the other small peak (M1) were not carried out.

Thus, it is presumed that hydroxylated piperidine derivatives such as 3-OH-Pip and 4-OH-Pip should be major candidates of the Pip metabolites excreted into urine. Both 3-OH-Pip and 4-OH-Pip were found not only in the urine of the Pip group but also in the urine of the control group. This is the first documentation that 3-OH-Pip and 4-OH-Pip are normal constituents in rat urine.

The study on pharmacological properties of 3-OH-Pip and 4-OH-Pip showed that both hydroxylated metabolites are pharmacologically different from Pip itself. As shown in Table 2, they do not have synaptotropic properties which constitute the most characteristic pharmacological feature of Pip (17, 19), but rather simulate the pharmacological actions of long-chained amino alcohols.

These findings suggest that Pip of either endogenous or exogenous origin is, at least in part, inactivated by being metabolized to its hydroxylated compounds and conjugated

| TABLE 2. Comparison of pharmacological actions of piperidine and 3- or 4-hydroxypiperidine |
|---------------------------------------------|-------|---------------|
| Respiratory stimulation                   | ++    | -             |
| Blood pressure rise                       | ++    | -             |
| Contraction of smooth muscle              | ++    | -             |
| Contraction of skeletal muscle            | ++    | -             |
| Prolongation of sleeping time             | ++    | +             |
| Decrease of spontaneous movements         | ++    | +             |
| Vomiting                                  | ++    | -             |
ones and is then excreted into the urine.

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REFERENCES


