

BIOCHEMICAL STUDIES ON PRECATORINE

(I) Effect of Tryptophan and its N-methyl Derivatives on Blood Sugar Level

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In 1956 Mirsky et al.⁽¹⁾ reported that the subcutaneous injection into normal rats of 4 millimoles of L-tryptophan per kilogram of body weight produced, 2 hours after administration, a blood sugar level of about 80% of the initial. It is also known that the administration of metabolic derivatives of L-tryptophan such as L-kynurenine, anthranilic acid, nicotinic acid, indole-3-acetic acid, 5-hydroxytryptophan or 5-hydroxytryptamine produces a significant hypoglycemia in normal rats⁽²⁾.

Precatorine (α -N, N-Dimethyl-L-tryptophan) is a new amino acid first isolated in our laboratory from the seeds of *Abrus precatorius*⁽³⁾. It is interesting to know that the chemical components of indole-containing amino acids in the jequirity seed also contain L-tryptophan, abrine (α -N-monomethyl-L-tryptophan)⁽⁴⁾ and hypaphorine (the betaine of L-tryptophan)⁽⁵⁾.

In this paper we will report the effect of three N-methyl derivatives—abrine, precatorine and hypaphorine on the blood sugar level of normal rats in comparison with the effect of L-tryptophan.

EXPERIMENTAL

Materials:

L-Tryptophan was purchased from Kanto Chemical Co., Inc., and abrine and precatorine were obtained from the alcohol extracts of the seeds of *Abrus precatorius*⁽⁶⁾. Abrine hydrochloride was prepared by adding concentrated hydrochloric acid to the alcohol solution of abrine. Hypaphorine nitrate was

synthesized according to the method of Van Romburgh and Barger⁽⁶⁾.

Methods:

The L-Tryptophan and its three N-methyl derivatives were separately dissolved in normal saline. The concentration of each solution was 10 mg. per ml. One ml. of the solution was injected subcutaneously to male rats, of Long-Evans strain, each weighing from 200 to 250 grams approximately. After an overnight fasting of the latter, blood samples were taken from the cut tails of the rats in 0.02 ml. Sahli pipette immediately before the injection and at intervals of 30 minutes for a duration of 4.5 hours thereafter. The control rats, which received only an injection of 1 ml. of normal saline, were tested to estimate blood sugar concentration in the same manner. Proteins and the bulk of the saccharoids were removed by precipitation with 1 ml. of 5% zinc sulfate and 1 ml. of 0.3N barium hydroxide reagent. After standing 5 minutes, the blood samples were centrifuged at 3000 RPM for 10 minutes. The blood sugar was determined according to the modified method of Folin and Wu⁽⁷⁾.

RESULTS AND DISCUSSION

The effect of the subcutaneous injection of 10 mg of L-tryptophan, abrine hydrochloride, precatorine or hypaphorine nitrate on blood sugar concentration is demonstrated in Tables 1, 2, 3 and 4 respectively. Table 5 indicates the blood sugar concentration of the control rats. The reduction of blood sugar con-

Table 1. The effect of the subcutaneous injection of tryptophan on blood sugar level of rats after overnight fasting

Exp. No.	Minutes after injection of 10 mg of tryptophan Blood sugar mg per 100 ml of blood									
	0	30	60	90	120	150	180	210	240	270
1	123.6	118.0	124.0	93.0	89.0	98.0	113.0	121.0	120.0	123.6
2	107.3	105.0	109.0	75.5	73.0	73.0	80.0	94.0	98.0	101.0
3	127.5	112.0	135.0	109.0	94.0	85.6	81.6	105.0	111.4	126.0
4	119.2	109.0	98.0	91.7	86.8	—	103.5	—	110.0	115.6
5	136.0	128.7	119.2	102.8	95.6	92.3	107.0	114.0	128.5	136.0
Average	122.7	114.5	116.6	94.4	87.7	87.2	97.0	108.5	113.6	120.4
% of initial	100	93.32	95.03	76.93	71.48	71.03	79.05	88.43	95.74	98.13

Table 2. The effect of the subcutaneous injection of abrine hydrochloride on blood sugar level of rats after overnight fasting

Exp. No.	Minutes after injection of 10 mg of abrine hydrochloride Blood sugar mg per 100 ml of blood									
	0	30	60	90	120	150	180	210	240	270
1	118.4	96.0	99.3	85.0	80.3	78.4	75.0	84.4	97.3	110.0
2	120.0	108.0	110.4	90.2	84.2	80.1	—	95.0	102.0	114.5
3	135.1	101.2	105.0	83.2	86.0	79.3	73.0	89.0	101.0	120.0
4	116.3	108.0	115.0	84.0	79.2	76.2	71.1	89.4	96.0	116.0
5	106.7	93.0	96.0	84.2	76.0	68.0	72.3	83.6	94.0	107.0
Average	119.3	101.2	105.1	87.3	81.1	76.4	72.9	88.3	98.1	113.5
% of initial	100	84.82	88.09	73.18	68.98	64.04	61.11	74.02	82.23	95.16

Table 3. The effect of the subcutaneous injection of precatorine on blood sugar level of rats after overnight fasting

Exp. No.	Minutes after injection of 10 mg of precatorine Blood sugar mg per 100 ml of blood									
	0	30	60	90	120	150	180	210	240	270
1	120.5	109.0	128.3	98.0	83.5	77.6	74.0	79.2	93.3	115.0
2	139.1	125.0	141.2	101.0	—	80.3	76.2	83.6	99.0	127.1
3	124.4	124.4	127.0	96.0	85.0	72.0	70.0	82.0	96.0	125.0
4	115.3	102.0	120.0	86.0	78.0	70.4	70.0	84.3	101.2	114.2
5	—	108.4	112.0	87.0	80.2	72.6	68.5	78.0	96.0	120.0
Average	124.8	113.8	125.7	93.6	81.7	74.6	71.8	81.4	97.1	120.3
% of initial	100	91.18	100.72	75.0	65.46	59.78	57.53	65.22	77.80	97.06

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Table 4. The effect of the subcutaneous injection of hypaphorine nitrate on blood sugar level of rats after overnight fasting

Exp. No.	Minutes after injection of 10 mg of hypaphorine nitrate Blood sugar mg per 100 ml of blood									
	0	30	60	90	120	150	180	210	240	270
1	130.6	118.0	130.0	—	93.4	82.0	77.2	85.0	105.0	125.8
2	112.0	104.0	119.0	83.0	77.2	72.0	68.3	80.0	98.2	110.5
3	124.0	98.0	122.8	92.4	81.0	76.0	72.4	84.0	99.0	124.0
4	134.4	114.0	114.0	102.0	92.0	84.1	76.0	89.0	104.0	134.4
5	128.0	112.0	126.0	89.6	84.4	74.0	70.3	82.0	106.0	126.0
Average	125.8	109.2	122.4	91.8	85.6	77.6	72.8	84.0	102.4	124.1
% of initial	100	86.8	97.3	72.97	68.04	61.69	57.87	66.77	81.40	98.65

Table 5. The effect of the subcutaneous injection of normal saline on blood sugar level of rats after overnight fasting

Exp. No.	Minutes after injection of 1 ml of normal saline Blood sugar mg per 100 ml of blood									
	0	30	60	90	120	150	180	210	240	270
1	123.6	123.6	123.6	119.0	118.7	123.6	117.5	111.3	120.0	122.0
2	117.4	110.7	134.2	105.5	116.0	—	110.8	137.1	—	125.0
3	127.5	127.0	136.0	122.4	120.8	125.0	—	124.8	124.8	122.0
4	139.1	139.1	136.5	139.1	127.5	139.0	134.3	135.9	133.9	132.0
5	142.9	126.3	148.0	133.5	139.2	—	132.0	139.0	136.0	138.0
Average	130.1	125.3	135.7	123.9	124.4	129.2	123.7	129.6	128.7	125.8

centration is expressed as a percentage of the initial. It is noted that the reduction of the blood sugar concentration occurs after the injection of L-tryptophan or its N-methyl derivatives. But 60 minutes after the injection the blood sugar concentrations have a little elevation as compared with those of the 30 minutes period, especially precatorine and hypaphorine nitrate. The reduction of the blood sugar concentration by L-tryptophan lasted for 150 minutes and reached a value of 71.03% of the initial and 180 minutes for abrine, precatorine and hypaphorine to about 61.11%, 57.53% and 57.87% of the initial respectively. After a 270 minute period, the blood sugar level returns to the initial value. With the control group of rats, the blood sugar level ranges from 95.08% to 104.30% of

the initial during the period of observation. The results in tables 1, 2, 3, 4 and 5 are summarized in Fig. 1.

According to the hypothesis of Mirsky^(1,2), the hypoglycemic action of L-tryptophan and its metabolic derivatives is due to their inhibitory effect on insulinase which destroys insulin. Mirsky⁽²⁾ also pointed out that some of the metabolic derivatives of L-tryptophan were effective as hypoglycemic agents which exert, in common, an effect on the growth of plants, namely: indole-3-acetic acid, anthranilic acid, 5-hydroxytryptamine, whether precatorine can act as plant growth regulators is not known. Tung and Cohen⁽³⁾ showed that abrine could be demethylated to tryptophan and formic acid in the rat liver by oxidation in the

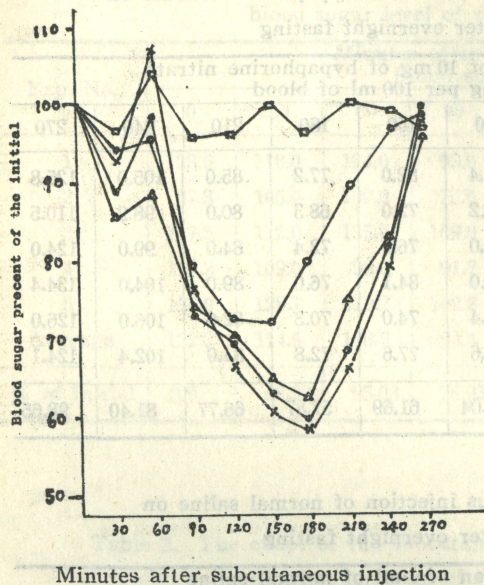


Fig. 1. The effect of the subcutaneous injection of 10 mg of L-tryptophan and its N-methyl derivatives on the blood sugar of rats after overnight fasting weighing from 200 to 250 grams as compared with normal rats.

Each mark indicated the average blood sugar level of the experiment.

- Rats received the injection of normal saline
- Rats received the injection of L-tryptophan
- △—△ Rats received the injection of abrine HCl
- ×—× Rats received the injection of precatorine
- Rats received the injection of hypaphorine-NO₂

presence of demethylase. The metabolism of precatorine and hypaphorine in the rat is being studied in our laboratory now.

From Fig. 1. it is known that the hypoglycemic effect of L-tryptophan and its three N-methyl derivatives is in the order of precatorine and hypaphorine, abrine, and L-tryptophan being the least effective. This may be due to the different metabolic fates

or the difference in the number of N-methyl groups of these four amino acids. It seems that the potency of the hypoglycemic action is directly related to the number of N-methyl groups present in them. As far as the same hypoglycemic effect of precatorine and hypaphorine is concerned, it may be due to their similar inhibitory effect on the insulinase. The studies to determine whether or not abrine, precatorine, and hypaphorine behave like L-tryptophan as an inhibitor of the insulinase is also in progress in our laboratory. It was to be noted that there were no abnormal phenomenon such as coma or convulsion observed in rats after the administration of 10 mg. of L-tryptophan, abrine hydrochloride, precatorine or hypaphorine nitrate.

SUMMARY

The subcutaneous administration of L-tryptophan or its three N-methyl derivatives to normal rats produced a significant reduction of blood sugar level. The hypoglycemic effect of these four amino acids is in the following order: precatorine and hypaphorine, abrine, and L-tryptophan being the least effective.

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Precatorine 之生化學研究

第一報：色氨酸及它的 N-甲基衍行物對於血糖之影響

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將 L-色氨酸或它的三種 N-甲基衍行物皮下注射於正常之大花鼠時，對於血糖會產生一有意義之降低，這四種氨基酸之降血糖作用是以 precatorine

和 hypaphorine 最大，abrine 次之，色氨酸再次之。

Method of isolation of the compound: The Penicillium islandicum grown in 250 ml. Petri-dishes was all transferred into 500 ml. Warburg flasks and was divided into 10 flasks. Incubation was obtained in the presence of 10% yeast extract and the filtrate (filtered broth) was used for the isolation of the compound.

(1) The residues: About 3 kg. of the residues were distilled with a water bath for 4 days. The extract was concentrated under reduced pressure to ca. 25 ml. This concentrate was passed through a Whatman No. 1 filter paper (Whatman No. 1, 30 cm. x 30 cm.) Chloroform was used as an eluent and the first

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It was known that there are three amino acids in the filtrate, the so-called yellowish-brown crystals, caused by Penicillium islandicum yellowish rice, caused by Penicillium islandicum 20pp. The poisonous substances of the first two were found to be citrullin and Citrullin, in the form of a yellow pigment of polyene, respectively. The toxic substances of the last one have been identified as alanine, methionine, and aspartic acid.

In this report it will be reported that there are three compounds isolated in crystals from Penicillium islandicum when cultured in the koji juice media. Two of the new crystals are obtained from the cultured mycelium and both possess fluorescence when viewed under ultraviolet light. One of the crystals, the koji A-1, was obtained from the cultured broth. The method of isolation and some properties of the compounds will be described in the following.

EXPERIMENTAL

Organism and Culture conditions: Penicillium islandicum (IC-200), obtained through the courtesy of Dr. C. F. Hen (Dean of Taipei Medical College), was used throughout this work.

Instead of Caspary's slant media, koji juice slant media was used for the growth of Penicillium islandicum. After the incubation at 30°C for 4 days the spores were transferred