DISTRIBUTION OF n-BUTYLBIGUANIDE-\(^{14}\)C HYDROCHLORIDE IN MOUSE TISSUES

YONG-JA YOH

Department of Pharmacology, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto

Received for publication February 13, 1967

Although the oral and parenteral administration of n-butylbiguanide in rabbits produces a hyperglycemic or hypoglycemic effect depending on the dose, the compound causes only a hypoglycemic response in normal as well as alloxan-diabetic rats (1). Clinical studies show a high incidence of gastrointestinal distress in patients as a remarkable side effect of the orally administered n-butylbiguanide (2). However, no pathohistological change in the gastrointestinal tract has been observed in rats after receiving the various doses of the compound for 6 months (1). Therefore, the anorexia and diarrhea of the diabetic patients treated with this compound are assumed to be functional in origin.

Experimental studies on the distribution of hypoglycemic biguanides-\(^{14}\)C by Kaneko (2), Wick et al. (3), Cohen et al. (4) and Beckmann (5) are consistent with each other in that biguanides distribute extensively to the gastrointestinal tract. The present experiments were designed to know the distribution of n-butylbiguanide-\(^{14}\)C in tissues and the nature of urinary metabolites in mice.

METHODS

Blood sugar level after n-butylbiguanide in mice

In a preliminary experiment, the doses of 50 and 200 mg/kg of n-butylbiguanide, which were ranging from subeffective to definitely hypoglycemic dose in normal rat (1),
were orally or subcutaneously given to intact male dd-mice (about 15 g), fasted for 20 hours but allowed free access to water. The details of the measurement of blood sugar level were described in the previous report (1). As shown in Fig. 1, the oral dose at 200 mg/kg produced a hypoglycemia to 60% of the original level at the maximum and it sustained for more than 4 hours. The fall of blood sugar level 2 hours after the subcutaneous injection of 50 mg/kg was nearly equal to the corresponding value after the oral dose of 200 mg/kg. Even the oral dose of 50 mg/kg lowered the blood sugar level to 80% of the original 2 hours after administration. Taking into consideration of the results on rats in the previous report (1), it is apparent that the mouse responds more sensitively than the rat to n-butylbiguanide with a hypoglycemia.

Estimation of $^{14}C_2$ in expired air

$n$-Butylbiguanide-$^{14}C$, labeled on two carbons of the biguanide group (specific activity 1.07 mc/g), was supplied by the Dainippon Pharmaceutical Co., Osaka. Using n-butylbiguanide as a carrier a stock solution of the labeled biguanide, 5 mc/20 mg/ml, was prepared and stored at $-4^oC$. Ten ml/kg of the solution was administered orally to mice. The treated mice were housed in the Weinhouse metabolic cage (6) and the expired air during 24 hours of the observation was absorbed into 2 N NaOH. A precipitate of BaCO$_3$, produced by the addition of excess 0.5 N Ba(OH)$_2$ to the CO$_2$-containing NaOH, was filtered, then washed three times with distilled water. The radioactivity of an appropriate amount of dried BaCO$_3$ powder on a planchet was determined by means of a Q-gas flow counter (Nuclear Chicago Counter: Model 181B, Gas flow detector: Model D-47).

Estimation of $n$-butylbiguanide-$^{14}C$ in blood

Animals were orally given with n-butylbiguanide-$^{14}C$ in dose of 50 mc/200 mg/kg, and sacrificed by head amputation 2 hours later. A 0.5 ml of arterial blood was collected, added with 2 ml of 5% HClO$_4$, stirred and centrifuged. The sediment thus obtained was washed with the same volume of 2% HClO$_4$ and again centrifuged. All the supernatants were collected and neutralized with the addition of 10 N KOH using phenol red as an indicator. The neutral solution was centrifuged, and a 0.2 ml of the supernatant after an appropriate dilution was placed on a planchet and dried for the determination of the radioactivity as described above.

Estimation of the radioactivity in urine and faeces

Urine and faeces were collected at the 1st, 2nd, 4th, 8th and 24th hour after the oral administration of 50 mc/200 mg/kg of n-butylbiguanide-$^{14}C$. A 0.2 ml of diluted urine was dried on a planchet for the estimation of total radioactivity. Faeces was homogenized with total 10 ml of 3.5% HClO$_4$ and the radioactivity was estimated as described above.

Distribution of $n$-butylbiguanide-$^{14}C$ in tissue

The doses of n-butylbiguanide-$^{14}C$ administered were 50 mc/200 mg/kg and 50 mc/50 mg/kg orally, and 50 mc/30 mg/kg subcutaneously. The first dose group was sacrificed
1, 2, 4, 8 and 24 hours later by head amputation under ether anesthesia. The other two groups were killed 2 hours after the drug administrations. Each group consisted of 6 mice and the chemical analysis was performed on animals pooled two each. Tissues to be analyzed were removed immediately after killing, washed gently 4 times with saline, then weighed. No radioactivity was detected in the last washings. The tissues were homogenized with 2-4 ml of 3.5% HClO₄ and the radioactivity was estimated as described.

Urinary excretion of n-butylbiguanide-¹⁴C

Urine samples collected 1, 2, 4 and 24 hours after the oral administration of 50 μc/200 mg/kg of n-butylbiguanide-¹⁴C were spotted on a sheet to filter paper (No. 50, Toyo Roshi). On another sheet, the mixtures of urine sample and standard n-butylbiguanide-¹⁴C were spotted. The spots were developed for 20 hours in a mixture of pyridine, iso-amylscohol and water in the ratio of 7:7:6. The radioactive metabolites on paper chromatogram were detected by means of a radioactivity detector (Actigraph II: Model C100B, Analytical count ratemeter: Model 1620B, Nuclear Chicago).

RESULTS

1. Radioactivity in expired air

Any radioactivity was undetected in CO₂ from the 24-hour expired air after oral administration of 50 μc/200 mg/kg of n-butylbiguanide-¹⁴C. It seems unlikely that the compound is subjected to an oxidative degradation of the biguanide group to produce ^¹⁴CO₂.

2. Blood concentration of n-butylbiguanide-¹⁴C

A considerable amount of the radioactivity was detected already 1 hour after the oral dose of 50 μc/200 mg/kg of n-butylbiguanide-¹⁴C, and the peak radioactivity was found at 2 hours. The amount of radioactive substance per ml blood at the peak was estimated as 28 μg/ml. Thereafter, the radioactivity declined gradually until the value of 11 μg/ml was attained at the 8th hour. At the 24th hour the amount of radioactive substance was only 1 μg/ml. The results are shown in Table 1.

<table>
<thead>
<tr>
<th>Hours after administration</th>
<th>n-Butylbiguanide μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>24</td>
<td>1</td>
</tr>
</tbody>
</table>

3. Radioactivity in urine and faeces

Radioactivity was detectable from urine already at the 1st hour of drug administration. As shown in Fig. 2, the percent urinary excretions of radioactivity, in reference to the administered, at 2, 8 and 24 hours were 25.88%, 52.84% and 87.65%, respectively. On the other hand, the percent excretion in faeces for 24 hours was only 5.5% of the administered radioactivity. This indicates that the orally administered n-butylbiguanide is absorbed almost completely from the gastrointestinal tract and excreted largely in urine.
4. Distribution of \(n\)-butylbiguanide-\(^{14}\)C in tissue

**Intestine:** As shown in Fig. 3, \(n\)-butylbiguanide-\(^{14}\)C distributed rapidly to the small intestine, and the radioactive substance detected 1 hour after the oral administration of 200 mg/kg was 181.8 \(\mu\)g/g tissue weight. The peak activity was found at the 2nd hour, coincident well with that in blood. At 8 hours a pronounced amount of 89.4 \(\mu\)g/g remain-
ed still in the intestine. The peak uptake to the large intestine was observed at the 4th hour. The total radioactivity in the small and large intestines 2 hours after the oral dose of 50 mg/kg was one-fourth of that after the oral dose of 200 mg/kg. A predominant distribution of the radioactivity to the intestines was also obtained 2 hours after the subcutaneous injection of 50 mg/kg, but the amount of radioactive substance was about half as much as that after the same dose by the oral route. It is likely that the circulating n-butylbiguanide is taken up by the intestine considerably and remained there for relatively long time.

Stomach: The maximum amount of radioactive substance in the stomach after the oral administration of 200 mg/kg was 192.0 μg/g at 1 hour. Then the amount declined gradually to 126.0, 119.4 and 41.4 μg/g, at 2, 4 and 8 hours, respectively. Two hours after the oral dose of 50 mg/kg, 22.4 μg/g of radioactive substance was present. On the other hand, the value of 7.1 μg/g in the stomach 2 hours after the subcutaneous injection of 50 mg/kg was low as compared with that in the intestine. The results are shown in Fig. 4. These findings indicate that the affinity of n-butylbiguanide to the stomach is less than that to the intestine.

**FIG. 4. Concentration of n-butylbiguanide in the stomach.**

Gastrointestinal contents: Because of a difficulty in weighing exactly all the gastrointestinal contents, only a percent value of radioactivity to the administered was calculated. The radioactivity in the gastric contents 1, 2, 4 and 8 hours after the oral dose of 200 mg/kg was 31.05%, 10.75%, 9.93% and 4.31%, respectively. On the other hand, 2 hours after the oral dose of 50 mg/kg, only 0.49% of the administered was found. A marked difference between the radioactivities in gastric contents after the two doses suggests that the administration of a large dose of n-butylbiguanide with a subsequent higher uptake by the stomach prolongs an emptying time of gastric contents to the intestine. On the subcutaneous injection of 50 mg/kg, 0.76% of the administered was found at 2 hours.
One, 2 and 4 hours after the oral dose of 200 mg/kg, the total radioactivities in the contents of both small and large intestines were 6.66%, 7.99% and 7.20% of the administered, respectively. The activity then declined to 0.74% at 24 hours. These facts again indicate a ready absorption of the compound from the intestines. The radioactivity in the intestinal contents after the oral dose of 50 mg/kg did not significantly differ from that after the oral dose of 200 mg/kg, and the activity after the subcutaneous dose of 50 mg/kg was about half as much as that after the oral administration of the same dose, suggesting that the circulating n-butylbiguanide was in some part excreted to the intestinal lumen. These are shown in Fig. 5 and Table 2.

Liver: The peak uptake to the liver, 1.35% of the total, was found 2 hours after the oral administration of 200 mg/kg. The value became relatively large, because of a high ratio of liver weight/body weight. As referred to the amount of radioactive substance per g liver tissue weight, only 60.6 µg/g was detected at 2 hours. The value declined to 25.8 µg/g at 8 hours and the radioactivity disappeared almost completely from the liver at 24 hours. Two hours after the oral dose of 50 mg/kg, 17.3 µg/g of radioactive substance was present. A similar value was obtained after the subcutaneous injection of 50 mg/kg. The results are shown in Fig. 6. It is indicated that the liver retains a smaller amount of n-butylbiguanide.

Skeletal muscle: The radioactivity in the gastrocnemius muscle 1 hour after the oral dose of 200 mg/kg was almost negligible. Then the activity increased to 49.2 and 58.8 µg/g at 2 and 4 hours, respectively, comparable to the value in the liver. However, the amount of radioactive substance in the muscle after the oral and subcutaneous doses of 50 mg/kg was about half or one-third as much as the corresponding value in the liver.
<table>
<thead>
<tr>
<th>Total Recovered</th>
<th>Percent of the Activity Given</th>
<th>**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>La</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Kidney</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lung</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Skin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** The table represents the distribution of \( ^{14}C \) labelled N-BUTYL-BIGUANIDE in mice after administration. The percentage of activity given is calculated for different organs and tissues. The values are not explicitly stated in the visible portion of the image, but the structure is consistent with a typical scientific table format.
Adrenals: Although a percent distribution of radioactive substance in the adrenal glands 2 hours after the oral dose of 200 mg/kg of n-butylbiguanide was only 0.02%, the amount per g tissue of 123.6 µg/g was somewhat large as compared with other tissues. Such value was maintained until 8 hours. Definite amounts of radioactive substance per g tissue were also detected after the oral or subcutaneous administration of 50 mg/kg, being 23.4 and 27.3 µg/g at 2 hours, respectively. Thus a preferential and long-lasting retaining of n-butylbiguanide was seen in the adrenal glands.

Kidney: A considerable amount of radioactivity was detected in the kidney after the oral administration of n-butylbiguanide, the peak being attained at 1 to 2 hours. Then the activity declined sharply. The radioactivity 2 hours after the subcutaneous injection of 50 mg/kg was relatively small, being about half as much as that after the oral administration. The difference is probably derived from a rapid distribution of n-butylbiguanide to tissues with a consequent early excretion through the kidney after the subcutaneous injection.

Pancreas: The uptake of radioactive n-butylbiguanide by the pancreas was relatively small, and the peak distribution of 72.6 µg/g was observed at 8 hours.

Brain: No radioactivity was detectable in the whole brain.

Others: The thymus, lung, heart, spleen and testis contained radioactive substance below 55 µg/g 2 hours after the oral administration of 50 µC/200 mg/kg of n-butylbiguanide-14C. The radioactivity was almost absent at 24 hours. Similar findings were obtained in the epididymal adipose tissue. An exceptional finding was obtained in the heart, where the radioactivity after the subcutaneous dose was higher than that after the oral dose. All the results are summarized in Table 2.

5. Identification of urinary excretion products of n-butylbiguanide

Paper chromatograms of urine, collected at the 1st, 2nd, 4th and 24th hour after the
oral administration of n-butylbiguanide-\(^{14}\)C, revealed the presence of several radioactive substances of different Rf value. The results are shown in Fig. 7. Approximately same amounts of unchanged n-butylbiguanide (Rf 0.55) and its metabolic product of Rf 0.45 (III) were detected at 1 and 2 hours. In addition, minute amounts of four other metabolites, Rf of which were 0.23, 0.35, 0.68 and 0.75 (I, II, IV and V), respectively, were found. A metabolite II increased at 2 hours. From the 4th hour a metabolite IV, instead of III, increased in urine and the amount was almost equal to unchanged n-butylbiguanide-\(^{14}\)C at 24 hours. The metabolites I, II, III and IV as well as the parent com-

Fig. 7. Radiopaperchromatogram of mouse urine after the oral administration of n-butylbiguanide-\(^{14}\)C.
pound showed the Sakaguchi reaction, indicative of the presence of guanidine group in a chemical structure.

DISCUSSION

The effective and rapid absorption of n-butylbiguanide from the gastrointestinal tracts was confirmed by the following facts: 1) a rapid appearance of radioactive substance in blood and tissues, 2) a high radioactivity in the gastrointestinal tracts and 3) only a minute radioactivity in faeces after the oral administration of n-butylbiguanide-14C. The results of other investigators (2-5) also indicate an uptake of large amounts of biguanides by the gastrointestinal tracts. Radioautographic studies are expected to give more information regarding the higher uptake and affinity of biguanides to the gastrointestinal tracts than to other tissues.

The time course of blood concentration of radioactive substance after the oral or parenteral administration of n-butylbiguanide-14C correlated well with that of the hypoglycemic response. The peak level of radioactive substance in blood, 28 \( \mu g/ml \), at 2 hours and a decline to 11 \( \mu g/ml \) at 8 hours corresponded well to the hypoglycemia to 60% and 80% of original level, respectively. Blood concentrations of the labeled compound after the oral administration of dimethylbiguanide-14C were found to be one-fifth as much as those after the same doses of n-butylbiguanide (2), and ten times the dose of n-butylbiguanide was required to produce a corresponding hypoglycemic response by dimethylbiguanide (7).

Since no radioactivity was detected in CO2 from the expired air after the administration of n-butylbiguanide-14C and also since all the urinary excretion products showed the Sakaguchi reaction, characteristic of the presence of guanidine group, it is unlikely that the compound subjected to a metabolic degradation on the guanidine group. One and 2 hours after the administration, the parent compound and a metabolite III, which probably corresponded with 3-hydroxybutylbiguanide reported by Beckmann (5), were mainly excreted in urine. Then the metabolite III was replaced by IV. However, taking into consideration of time course, it is improbable that IV is responsible for the hypoglycemic effect of n-butylbiguanide. The other three metabolites should not be main metabolites.

Controversial results (2-4) were reported regarding the distribution of radioactive biguanides in the liver. The tissue concentration of the radioactive substance in the liver was not prominent, compared with other tissues such as the thymus, heart, kidney, skeletal muscle and epididymal adipose tissues. Such a poor affinity of the biguanide to the liver would deny the opinion proposed by Wick et al. (3) that the biguanides have a main site of action in the liver.

The facts that n-butylbiguanide was not taken up by the brain cause us to assume that a central mechanism would not take part in the hypoglycemia due to the compound and that the proliferation of the eosinophile cells in the anterior hypothalamus produced by chronic feeding of the biguanide (1) might be of peripheral origin.
Although the distribution of n-butylbiguanide in the pancreas was relatively small, the tissue concentration in the adrenal glands was two times as that in the liver. Söling (8) reported that the hypoglycemic action of n-butylbiguanide is exerted in adrenalectomized rats more extensively than in normal rats, and that the action is inhibited by a large amount of desoxycorticosterone or prednisolone. These facts suggest a role of the adrenals played in the hypoglycemic response to the biguanide. Proliferative changes of the fascicular zone in the adrenal cortex after chronic feeding of n-butylbiguanide (1) also might be correlated with the dense distribution of the compound in the adrenals.

**SUMMARY**

1. The oral and parenteral administrations of n-butylbiguanide lowered blood sugar level in mice.
2. No radioactivity was detected in CO₂ from the expired air at least for 24 hours after the oral administration of n-butylbiguanide-¹⁴C.
3. In addition to the unchanged parent compound, five metabolites were detected paper-chromatographically in urine.
4. Considerable amounts of n-butylbiguanide were retained in blood at least for 8 hours, and the time course of the blood concentration accorded with that of the hypoglycemic response.
5. The distribution of radioactivity, in terms of µg/g tissue weight, was most dense in the gastrointestinal tracts, followed by the adrenal glands. The radioactivity in the brain was almost negligible. The uptake of the compound by other tissues was uniform but small.

**REFERENCES**