Abstract—Various drugs known to bind to serum albumin were examined to determine whether or not they influenced the level of free tryptophan in serum in vitro and in vivo. Possible relationships between the serum free tryptophan level and serotonin (5-HT) synthesis in the brain and the hypothermic effects of these drugs were investigated. Of the drugs examined, sodium salicylate, sodium benzoate and indomethacin caused a significant increase in the concentration of serum free tryptophan and stimulated the synthesis of 5-HT in the brain. Hypothermia induced by salicylate and indomethacin was potentiated by pretreatment with pargyline, a monoamine oxidase inhibitor. Administration of benzoate did not cause any change in body temperature, but after pargyline a hypothermia did occur. However, pretreatment with parachlorophenylalanine, an inhibitor of 5-HT synthesis, did not influence the hypothermia induced by salicylate and indomethacin. Relationship between the hypothermic effect and the increase of 5-HT synthesis in the brain after a large dose of salicylate and indomethacin is discussed.

The rate of synthesis of 5-HT in the brain is known to be regulated principally by the availability of the precursor amino acid, L-tryptophan (1). Recently, it was shown that changes in the level of free, but not total tryptophan, in the plasma are well correlated with change in the brain tryptophan (2). L-Tryptophan is the only amino acid bound to serum albumin, and a wide variety of compounds, including salicylate, have been found to compete with L-tryptophan for this binding site on serum albumin (3, 4). It is possible that certain drugs which bind to serum albumin may also compete with tryptophan for its binding site; consequently, increasing the level of tryptophan in the serum and thereby increasing the synthesis of 5-HT in the brain.

In this work, we examined the effects of various drugs which bind to serum albumin on the ratio of free to bound tryptophan in rat serum in vitro and on 5-HT synthesis in the brain in vivo. The relationship between the synthesis of 5-HT in the brain and the hypothermic effect of some drugs was also examined.

MATERIALS AND METHODS

Animals: Male Sprague-Dawley rats, weighing 160 to 220 g, were decapitated between 13:00 and 15:00 hr. The brain was quickly removed, homogenized and the concentrations of tryptophan, 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were measured. Serum for analyzing tryptophan was obtained by the centrifugation of blood after clotting then stored at -20°C until analyzed.
Measurement of tryptophan in the serum and brain: Tryptophan in the serum and brain was assayed fluorometrically by the method of Denkla and Dewey (5). Serum free tryptophan was determined using 0.1 ml samples of ultrafiltrates, prepared by centrifuging 1 ml of serum in dialysis tubing (Size 8/32, Visking Co.) at 3,000 g for 2 hr at 0-4°C. 

Studies on rat serum in vitro: Sodium salicylate, sodium benzoate, sulfathiazole, aspirin, atropine sulfate, and pento- and phenobarbital sodium were dissolved in 0.9% NaCl. Indomethacin, phenylbutazone and aminopyrine were dissolved in a NaOH solution and diluted with 0.9% NaCl. Next, 0.1 ml of the test drug solution was added to 0.9 ml of normal rat serum and the mixture was analyzed for free tryptophan. As controls, 0.1 ml of saline or dilute alkali was added in place of the drug solution. The final pH range of these mixture was 6.5 to 8.0, in which only a negligible change of free tryptophan level was observed.

Measurements of 5-HT and 5-HIAA: Brain 5-HT was determined by the method of Bogdanski et al. (6) and brain 5-HIAA by the method of Curzon and Green (7) and Udenfriend et al. (8).

Measurements of body temperature: Body temperature was measured by inserting a thermistor probe about 5 cm into the rectum. All experiments were conducted at a room temperature of between 20 and 22°C.

RESULTS

Effects of various drugs on the ratio of free to total tryptophan in rat serum in vitro

Fig. 1 shows the in vitro effects of various drugs, known to bind to serum albumin, on the ratio of free to total tryptophan in rat serum. The drugs were tested at final concentrations of 2 mM; and of those examined indomethacin, salicylate, benzoate, sulfathiazole and phenylbutazone significantly increased the serum concentration of free tryptophan level was observed.

![FIG. 1. Effects of various drugs on the ratio of free to total tryptophan in rat serum in vitro.](image)

Drugs were added to serum at a concentration of 2 mM. Values are means of results in 4-8 experiments. * Significantly different from control (P<0.01).
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Phenylbutazine, aspirin, pento- and phenobarbital and atropine had no effect at this concentration. Linear increases in the concentration of free tryptophan were obtained with concentration ranges of 0.5 to 3.0 mM salicylate and indomethacin (data not shown).

Effects of various drugs on the concentration of serum total and free tryptophan in vivo

Table 1 shows the levels of total and free tryptophan 2 hr after administration of salicylate, benzoate and indomethacin. Salicylate and benzoate (300 mg/kg) significantly decreased the total tryptophan level and increased the free tryptophan level in the serum. Indomethacin, at a higher dose (100 mg/kg), significantly decreased the total tryptophan and increased the free tryptophan in the serum.

| Treatment       | Serum tryptophan (µg/ml) | Total | Free       |
|-----------------|--------------------------|-------|------------|
| Control         | (6)                      | 24.8 ± 2.0 | 2.0 ± 0.1 |
| Na salicylate   | (4)                      | 11.2 ± 1.8* | 9.5 ± 0.5* |
| Na benzoate     | (4)                      | 13.2 ± 2.3* | 10.9 ± 0.8* |
| Indomethacin    | (5)                      | 12.4 ± 0.8* | 3.5 ± 0.3** |

Sodium salicylate and sodium benzoate (300 mg/kg), and indomethacin (100 mg/kg) were administered i.p. 2 hr before sacrifice.

* Significantly different from control (P < 0.01).
** Significantly different from control (P < 0.05).

Effects of various drugs on the concentrations of tryptophan, 5-HT and 5-HIAA in rat brain

Table 2 shows the effects of intraperitoneal injection of aminopyrine and of the drugs which caused an increase in the serum free tryptophan level in vitro on the concentrations of brain tryptophan, 5-HT and 5-HIAA in rats. At the doses indicated in the table, sali-
FIG. 2. Time-course of changes in the concentrations of brain tryptophan, 5-HT and 5-HIAA after administration of salicylate.
Sodium salicylate (300 mg/kg) was administered i.p. at 0 hr.
Points and vertical bars show means ± S.E. of values in 4-6 rats.

Sodium salicylate and benzoate increased the concentrations of tryptophan, 5-HT and 5-HIAA in the brain 2 hr after their administration. Indomethacin, the drug which caused the greatest increase in free tryptophan in vitro, did not influence the concentrations of these three indole compounds at a dose of 20 mg/kg. However, at a dose of 100 mg/kg, indomethacin

FIG. 3. Effects of PCPA pretreatment on the body temperature responses to benzoate, salicylate and indomethacin administration in rats. Responses to sodium benzoate, (○) 300 mg/kg i.p.; (×) 300 mg/kg i.p. after PCPA. Responses to sodium salicylate, (△) 100 mg/kg i.p.; (○) 300 mg/kg i.p.; (●) 300 mg/kg i.p. after PCPA. Responses to indomethacin, (△) 20 mg/kg i.p.; (○) 100 mg/kg i.p.; (●) 100 mg/kg i.p. after PCPA. Drugs were injected at arrow. Values are mean differences between the rectal temperature of the saline-treated control and experimental groups. Each group included 4 to 8 rats. Vertical bars represent S.E. of mean values.
caused a significant increase in the brain 5-HIAA concentration. Phenylbutazone, sulfathiazole and aminopyrine had no effect at a dose of 100 mg/kg.

Fig. 2 shows the time-courses of changes in the levels of brain tryptophan, 5-HT and 5-HIAA after administration of salicylate. The concentration of brain tryptophan increased gradually for 2 hr, while those of 5-HT and 5-HIAA were maximal after 1 hr.

Effect of salicylate, benzoate and indomethacin on the concentration of brain 5-HT and on the body temperature

Figs. 3 and 4 show the effect of pargyline, a monoamine oxidase inhibitor (MAOI), and p-chlorophenylalanine (PCPA), a tryptophan-5-hydroxylase inhibitor, on the changes of rat body temperature induced by salicylate, benzoate and indomethacin.
TABLE 3. Effects of salicylate, benzoate and indomethacin on the concentration of brain 5-HT in rats pretreated with pargyline or p-chlorophenylalanine.

| Treatment         | Brain 5-HT (μg/g) |
|-------------------|------------------|
| Control           | 0.54±0.04        |
| Na salicylate     | 0.72±0.05        |
| Na benzoate       | 0.80±0.07        |
| Indomethacin      | 0.62±0.03        |
| Pargyline         | 0.91±0.02        |
| + Na salicylate   | 1.41±0.06        |
| + Na benzoate     | 1.52±0.12        |
| + Indomethacin    | 1.33±0.06        |
| PCPA              | 0.19±0.08        |
| + Na salicylate   | 0.22±0.09        |

Sodium salicylate (300 mg/kg), sodium benzoate (300 mg/kg) and indomethacin (100 mg/kg) were administered i.p. 2 hr before sacrifice. Pargyline-HCl (75 mg/kg) and PCPA (300 mg/kg) were administered i.p. 15 min and 48 hr respectively, before treatments with the drugs. Values are means±S.E. *Significantly different from the respective saline, pargyline or PCPA-treated group (P<0.01).

neal injection of salicylate or indomethacin caused a dose-dependent decrease in body temperature, while benzoate did not cause any change (Fig. 3). Salicylate, 300 mg/kg, and indomethacin, 100 mg/kg, alone produced maximal falls in temperature of 1.6±0.3°C, 1.5 hr after injection, and 1.5±0.3°C, 2.5 hr after injection, respectively. Pretreatment with 75 mg/kg of pargyline, which showed a fall in the temperature alone, intensified the hypothermia caused either by salicylate or by indomethacin (Fig. 4). Although sodium benzoate, 300 mg/kg did not influence the body temperature, a significant hypothermia was seen by the pretreatment of pargyline. Concomitantly with the potentiation of hypothermia responses, pretreatment with pargyline induced signs of remarkable excitation in animal behavior, including the rat treated with sodium benzoate. Salivation, vocalization, exophthalmos, tremor and head twitching occurred.

On the other hand, pretreatment with PCPA, 300 mg/kg i.p. 48 hr prior to experiments, did not influence the hypothermic effects of salicylate and indomethacin (Fig. 3).

Table 3 shows the contents of brain 5-HT after these treatments. The increases in 5-HT in the brain caused by salicylate, benzoate and indomethacin were enhanced by pretreatment with pargyline, whereas PCPA pretreatment blocked the increase in brain 5-HT by salicylate.

DISCUSSION

It has been shown that the rate of brain 5-HT synthesis is regulated principally by the availability of the precursor amino acid, L-tryptophan. This amino acid is normally present at a concentration considerably below the Km for tryptophan hydroxylase (1). Furthermore, it is thought that the serum free tryptophan concentration regulates the
level of brain tryptophan and consequently the rate of brain 5-HT synthesis (2). Recently, Tagliamonte et al. and other investigators reported that sodium salicylate (9), acetylsalicylate (10) and clofibrate (11) stimulated brain 5-HT synthesis by conversion of bound tryptophan in the serum to the free form. Our results showed that both in vitro and in vivo salicylate, benzoate and indomethacin displaced tryptophan from its binding site on serum albumin and increased the concentrations of tryptophan and 5-HIAA in rat brain. These results further support the hypothesis that free tryptophan in the serum controls the brain tryptophan concentration and hence brain 5-HT synthesis. Our results also indicate that neither phenylbutazone nor sulfathiazole increases the concentrations of tryptophan and 5-HIAA in the brain. This is probably because they have weak actions in displacing tryptophan from serum albumin (Fig. 1).

It has been found that tryptophan administration to rats pretreated with a MAOI resulted in marked excitatory behavior (12). This is thought to be due to an excessively elevated concentration of brain 5-HT. The excitation of animals treated with salicylate, benzoate or indomethacin after pretreatment with pargyline is identical with a behavioral change caused by administration of tryptophan following a MAOI. This seems to be the result of the same mechanism, from excessive elevation of the 5-HT concentration in the brain (Table 3).

5-HT has been shown to play an important part in regulating body temperature. In rats, the injection of 5-HT into the cerebral ventricles results in hypothermia (13). Baird and Lang (14) recently reported that the hypothermia induced by anesthetics was potentiated in rats and rabbits by the intraperitoneal or intraventricular injection of MAOI. They suggested that the anesthetics may release 5-HT and/or noradrenaline in the hypothalamus, and that inhibition of the metabolism of either or both these amines by MAOI could lead to potentiation of the temperature response to the anesthetics. Furthermore, it has been reported that short-term ether anesthesia increases the concentration of brain tryptophan and 5-HT synthesis (15). Satinoff showed that high doses of salicylate lowered the body temperature of rats kept in a 23°C environment (16). Our experiments also showed that high doses of salicylate or indomethacin cause hypothermia in rats kept at a room temperature of between 20 and 22°C. Furthermore, we recognized that the fall in the body temperature resulting from administration of these drugs was potentiated by pargyline, and that although benzoate alone had no effect on the body temperature, it caused hypothermic response after pargyline. This potentiation of hypothermia appears to be due to an excessive increase in the amount of brain 5-HT. However, the administration of benzoate, similar to the actions of salicylate and indomethacin, increased the concentrations of brain tryptophan, 5-HT and 5-HIAA, and pretreatment with PCPA did not inhibit the hypothermia induced by salicylate or indomethacin. These results suggest that the hypothermia induced by salicylate or indomethacin is not due to an increased 5-HT synthesis in the brain.
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