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MECHANISMS OF ELEVATION OF RAT BRAIN TRYPTOPHAN CONCENTRATION BY VARIOUS DOSES OF SALICYLATE

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1 The roles of inhibition of liver tryptophan pyrrolase activity and of displacement of tryptophan from its binding sites on serum proteins have been investigated in relation to the increase in rat brain tryptophan concentration after administration of various doses of sodium salicylate.

2 The elevation of brain tryptophan concentration by sodium salicylate (0.5 mg/kg) was caused by inhibition of liver pyrrolase activity, whereas that by doses of the drug of 50 mg/kg and above was achieved mainly by tryptophan displacement. Both tryptophan displacement and pyrrolase inhibition caused the increase in brain tryptophan concentration by sodium salicylate at 10 mg/kg.

3 The smallest dose of salicylate capable of displacing serum-protein-bound tryptophan was 2.5 mg/kg.

Introduction

Salicylate displaces tryptophan from its binding sites on serum proteins *in vitro* (McArthur & Dawkins, 1969) and *in vivo* in man (Smith & Lakatos, 1971) and rat (Badawy & Smith, 1972), and the resultant increase in the availability of circulating free tryptophan to the brain leads to an enhancement of cerebral 5-hydroxytryptamine synthesis (Tagliamonte, Biggio, Vargiu & Gessa, 1973; Bourgoin, Faivre-Bauman, Benda, Glowinski & Hamon, 1974). The elevation of rat brain tryptophan concentration was demonstrated in these studies with doses of sodium salicylate of 50–450 mg/kg body wt. Brain tryptophan concentration has recently been shown to be elevated by doses of the drug of 10 mg/kg (Badawy & Evans, 1981) and 0.5 mg/kg (A. A.-B. Badawy & M. Evans, unpublished observation), which were also found to inhibit liver tryptophan pyrrolase activity. It was therefore considered of interest to find out whether the elevation of brain tryptophan concentration by these latter doses is caused by pyrrolase inhibition, tryptophan displacement or both, and also to determine the smallest dose of the drug capable of displacing serum-protein-bound tryptophan.

Methods

Locally bred male Wistar rats (150–170 g) were housed three per cage (at 22 ± 1°C under natural light-dark cycles) and were maintained on cube diet 41B (Oxoid, Basingstoke, Hants) and water. The animals received an intraperitoneal injection of either sodium salicylate (0.5–400 mg/kg) or an equal volume (2 ml/kg) of 0.9% w/v NaCl solution (saline). The animals were killed between 13 h 00 min and 14 h 00 min either by stunning and cervical dislocation (for the determination of tryptophan pyrrolase activity in fresh-liver homogenates) or by decapitation (for all other determinations). Liver tryptophan pyrrolase activity and the concentrations of free (ultrafiltrable) serum, total (acid-soluble) serum and brain tryptophan were determined as described by Badawy, Evans & Punjani (1981). Tryptophan pyrrolase activity was determined either in the absence (holoenzyme activity) or in the presence (total enzyme activity) of added (2 μM) cofactor (haematin). The apoenzyme activity was obtained by difference. The holoenzyme and apoenzyme are respectively the haem-containing and haem-free forms of tryptophan pyrrolase in rat (and human) liver, and the total enzyme activity is the sum of those of the above two forms. Statistical analysis of results was performed by use of Student's *t* test. Chemicals were purchased from standard suppliers.

Results

The results in Table 1 show that all three activities of rat liver tryptophan pyrrolase were significantly inhibited in a dose-dependent fashion at 2 h after administration of doses of sodium salicylate of 0.5–50 mg/kg. However, the 400 mg/kg dose did not significantly alter the holoenzyme activity, but inhibited those of the total enzyme and apoenzyme by 59% and 92% respectively.

Both brain and free serum tryptophan concentra-

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Table 1 Effects of various doses of sodium salicylate on rat liver tryptophan pyrrolase activity and serum and brain tryptophan concentrations

Determination	*Dose of sodium salicylate* (mg/kg)				
Pyrrolase activity:	0	0.5	10	50	400
Holoenzyme	2.70 ± 0.26	1.50 ± 0.05**	1.20 ± 0.07**	0.80 ± 0.05***	2.30 ± 0.15
Total enzyme	6.30 ± 0.05	3.20 ± 0.06***	2.80 ± 0.21***	1.70 ± 0.09***	2.60 ± 0.09***
Apoenzyme	3.60 ± 0.21	1.70 ± 0.02***	1.60 ± 0.19***	0.90 ± 0.05***	0.30 ± 0.13***
Brain Trp	1.87 ± 0.07	2.29 ± 0.01***	2.84 ± 0.10***	3.23 ± 0.10***	5.53 ± 0.29***
Free serum Trp	1.54 ± 0.03	1.86 ± 0.04**	2.44 ± 0.05***	2.74 ± 0.06***	8.60 ± 0.23***
Total serum Trp	32.12 ± 0.64	39.32 ± 1.58**	36.68 ± 1.62†	23.48 ± 1.10***	21.56 ± 0.98***
Free serum Trp (%)	4.79 ± 0.08	4.73 ± 0.09	6.65 ± 0.27***	11.67 ± 0.26***	39.89 ± 1.95***

Rats received either sodium salicylate (0.5–400 mg/kg, i.p.) or an equal volume (2 ml/kg) of saline and were killed either 2 h later (for the determination of liver tryptophan pyrrolase activity) or 3.5 h later (for all other determinations). Pyrrolase activity is in μmol of kynurenine formed/h and per g wet wt. of liver (means ± s.e.mean for each group of 4). whereas all other determinations [except the free serum tryptophan (Trp) (%)] are in μg/ml of serum or per g wet wt. of brain (means ± s.e.mean for each group of 5). The values observed in salicylate-treated rats were compared with those obtained in the saline-treated controls, and the significance of differences is indicated as follows: †$P < 0.05$; **$P < 0.005$; ***$P < 0.001$.

tions were significantly elevated in a dose-dependent fashion at 3.5 h after administration of doses of salicylate of 0.5–400 mg/kg (Table 1). Total serum tryptophan concentration was also increased by the 0.5 mg/kg and 10 mg/kg doses of the drug, but not by the other two larger doses, which decreased it by 27% and 33% respectively. The percentage free serum tryptophan (an expression of tryptophan binding to serum proteins) was not significantly altered by the 0.5 mg/kg dose, but was increased by the other three doses, by 39, 144 and 733% respectively.

The results of the experiments to determine the smallest dose of sodium salicylate capable of displacing serum-protein-bound tryptophan, are shown in Table 2. At 1 h, both free serum and total serum tryptophan concentrations were equally increased by the 0.5 mg/kg and 1 mg/kg doses of the drug, by 10% and 18–21% respectively. The binding of tryptophan to serum proteins was therefore not significantly altered by the above two small doses of salicylate. By contrast, the percentage free serum tryptophan was significantly increased in a dose-dependent fashion by doses of the drug of 2.5–10 mg/kg. Under these latter conditions, free serum tryptophan concentration was significantly increased, whereas that of total serum tryptophan was either not significantly different from that of controls (after administration of the 2.5 mg/kg dose), or was significantly decreased (by the two larger doses).

Discussion

Inhibition of liver tryptophan pyrrolase activity leads

Table 2 Effects of various doses of sodium salicylate on serum tryptophan concentrations and binding in the rat

Dose of sodium salicylate	*Serum tryptophan concentration* (μg/ml)		*Free serum tryptophan*
(mg/kg)	Free	Total	(%)
0	1.45 ± 0.05	24.96 ± 0.79	5.81 ± 0.19
0.5	1.59 ± 0.03†	27.40 ± 0.68†	5.80 ± 0.22
1	1.75 ± 0.04**	29.56 ± 1.45‡	5.92 ± 0.31
2.5	1.67 ± 0.08†	25.74 ± 1.21	6.49 ± 0.19†
5	2.32 ± 0.04***	23.02 ± 0.62†	10.08 ± 0.49***
10	2.69 ± 0.04***	20.90 ± 0.85*	12.87 ± 0.61***

Rats received either sodium salicylate (0.5–10 mg/kg, i.p.) or an equal volume (2 ml/kg) of saline and were killed 1 h later. Values are means ± s.e.mean for each group of 5. The values observed in salicylate-treated rats were compared with those obtained in the saline-treated controls, and the significance of differences is indicated as follows: †$P < 0.05$; ‡$P < 0.025$; *$P < 0.01$; **$P < 0.005$; ***$P < 0.001$.

to proportionate increases in free serum and total serum tryptophan concentrations without altering tryptophan binding to serum proteins (see e.g. Badawy *et al.*, 1981). The similar results obtained with sodium salicylate (0.5 mg/kg) (Table 1) therefore strongly suggest that this small dose increases brain tryptophan concentration by inhibiting liver tryptophan pyrrolase activity, but not by displacing tryptophan from its binding sites on serum proteins. However, the 10 mg/kg dose increased brain tryptophan concentration by both mechanisms, because it both decreased tryptophan binding to serum proteins and increased total (as well as free) serum tryptophan concentration (Table 1). A distinction between these two mechanisms cannot be made in relation to the elevation of brain tryptophan concentration by the 50 mg/kg or 400 mg/kg doses, because of their ability to lower total serum tryptophan concentration; an effect that has previously been observed (Badawy & Smith, 1972) and which could be explained by increased tissue (particularly liver) uptake and catabolism. These latter two doses increased brain tryptophan concentration probably by their strong displacement of protein-bound tryptophan, although pyrrolase inhibition cannot be ruled out entirely.

It will be seen from the results in Table 1 that sodium salicylate (400 mg/kg) did not inhibit the pyrrolase holoenzyme activity at 2 h. This absence of inhibition has previously been shown (Badawy & Smith, 1971) to be a transitory recovery of the inhibited enzyme before the occurrence of a major tryptophan-mediated enhancement, which started at 3 h. The 50 mg/kg dose was also previously shown (Badawy & Smith, 1972) not to alter the holoenzyme activity at 3 h.

The fact that sodium salicylate (0.5 mg/kg) does not displace bound tryptophan is further suggested by the observation that 2.5 mg/kg was the smallest dose capable of causing such displacement (Table 2). This latter dose is approximately one-half of an aspirin tablet and it is therefore remarkable that brain tryptophan concentration can be elevated by such a small dose, and, even more surprisingly, by the 0.5 mg/kg dose. Traces of salicylate can therefore alter tryptophan disposition and this illustrates the need for strict control of aspirin intake in studies of tryptophan metabolism.

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References

BADAWY, A. A.-B. & EVANS, M. (1981). Inhibition of rat liver tryptophan pyrrolase activity and elevation of brain tryptophan concentration by administration of antidepressants. *Biochem. Pharmac.*, **30,** 1211–1216.

BADAWY, A. A.-B., EVANS, M. & PUNJANI, N.F. (1981). Reversal by naloxone of the effects of chronic administration of drugs of dependence on rat liver and brain tryptophan metabolism. *Br. J. Pharmac.*, **74,** 489–494.

BADAWY, A. A.-B. & SMITH, M.J.H. (1971). The effects of salicylate on the activity of rat liver tryptophan pyrrolase *in vitro* and *in vivo*. *Biochem. J.*, **123,** 171–174.

BADAWY, A. A.-B. & SMITH, M.J.H. (1972). Changes in liver tryptophan and tryptophan pyrrolase activity after administration of salicylate and tryptophan to the rat. *Biochem. Pharmac.*, **21,** 97–101.

BOURGOIN, S., FAIVRE-BAUMAN, A., BENDA, P., GLOWINSKI, J. & HAMON, M. (1974). Plasma tryptophan and 5-HT metabolism in the CNS of the newborn rat. *J. Neurochem.*, **23,** 319–327.

McARTHUR, J.N. & DAWKINS, P.D. (1969). The effects of sodium salicylate on the binding of L-tryptophan to serum proteins. *J. Pharm. Pharmac.*, **21,** 744–750.

SMITH, H.G. & LAKATOS, C. (1971). Effects of acetylsalicylic acid on serum protein binding and metabolism of tryptophan in man. *J. Pharm. Pharmac.*, **23,** 180–189.

TAGLIAMONTE, A., BIGGIO, G., VARGIU, L. & GESSA, G.L. (1973). Increase of brain tryptophan and stimulation of serotonin synthesis by salicylate. *J. Neurochem.*, **20,** 909–912.

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