percentage reduction in responses to a standard dose of noradrenaline given before and after 20 min exposure to SY28 (with and without varying doses of amino-acid).

Results showed that the amino-acids, histidine, phenylephrine and tyrosine, exerted some slight degree of protection against blockade by SY28, but the only significant result was the dose-dependent effect of the amino-acid tryptophan. As the dose of tryptophan was increased, the degree of irreversible blockade by SY28 against noradrenaline was decreased (Fig. 1). The low solubility of (−)-tryptophan prevented experimentation using doses in excess of 4×10^{-3} g/ml.

There are two possible explanations to account for the action of tryptophan under these conditions. The amino-acid may be able to occupy the aromatic sub-site, normally occupied by SY28, within the alpha receptor and therefore compete with SY28 for that site. The other possibility is that tryptophan provides an alternative to the aromatic sub-site of the alpha receptor, therefore SY28 is preferentially bound to tryptophan, rather than the alpha receptor, when sufficient molecules of the amino-acid are available in the bathing fluid. Interaction between ^{14}C-SY28 and amino-acids, including to a small extent tryptophan, has been shown *in vitro*.[6]

It is therefore tempting to speculate that, assuming the alpha receptor is proteinaceous in nature, for which there is considerable evidence, the aromatic site within the alpha receptor, to which SY28 binds, is in fact tryptophan.

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Evidence for a peripheral effect of fusaric acid, a dopamine β-hydroxylase inhibitor, on serotonin metabolism

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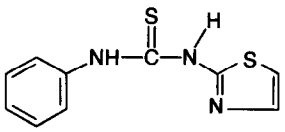
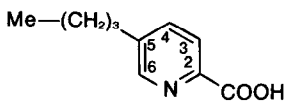
FUSARIC acid (5-butylpicolinic acid) has been shown to be a potent inhibitor of dopamine-β-hydroxylase (DBH)[1]. Johnson *et al.* have described another group of DBH inhibitors, the most studied being 1-phenyl-3-(2-thiazolyl)-2-thiourea (U-14,624)[2].

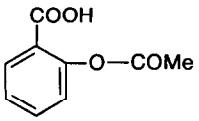
Besides depleting the brain norepinephrine (NE) stores, these two inhibitors influence the brain 5-hydroxytryptamine (5-HT) metabolism, increasing the cerebral levels of tryptophan (TRY) and 5-hydroxyindole acetic acid (5-HIAA) with a slighter elevation of 5-HT itself.



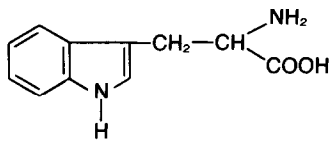
U-14,624



Fusaric acid



Acetylsalicylic acid



Tryptophan

The present study was designed to investigate the extra- and intra-cerebral tryptophan metabolism simultaneously after treatment by DBH inhibitors and to compare the mechanisms of action of fusaric acid and U-14,624.

MATERIALS AND METHODS

Fusaric acid was obtained from Banyu Pharmaceutical Co. Ltd., 2-chrome, Nihonbashi Honcho, Chuo-Ku, Tokyo, Japan. U-14,624 was purchased from Aldrich Chemical Co. Inc., Milwaukee, Wis., U.S.A.

Fusaric acid was dissolved in distilled water and the pH brought to 5·5 with sodium hydroxyde before making up to the final volume. U-14,624 was suspended in 0·25% aqueous methyl cellosolve.

Male Sprague–Dawley rats weighing 275 ± 25 g were starved overnight and injected intraperitoneally with 100 mg/kg of one of these drugs in a volume of 0·5 ml and killed by decapitation after 3 hr. Control rats received 0·5 ml of saline. Each group consisted of ten animals. Blood samples were collected for total and free tryptophan determination and the brains removed to measure either catecholamines or 5-HT metabolism.

For catecholamines assay, the brains were immediately homogenized in 0·4 N perchloric acid and, after centrifugation, the supernatant was frozen until assayed according to Comoy and Bohuon.[3] Another group of brains was homogenized in acidified *n*-butanol with an Ultra-Turrax (3 times 5 sec). Tryptophan, 5-HT, 5-HIAA were determined in the same brain extract.[4,5]

Serum ultrafiltrates for free tryptophan determination were obtained quickly after filtration through a Centriflo membrane cone (CF 50 Amicon) by centrifugation (30 min at 1000 *g*).

Total and free serum tryptophan was assayed according to Hess and Udenfriend.[6]

RESULTS AND DISCUSSION

The results are summarized in Table 1. The inhibitory effect of U-14,624 and fusaric acid on DBH is shown by a sharp drop in the brain NE concentrations. The brain dopamine (DA) level is not significantly changed by administration of 100 mg/kg of the drugs and a 3 hr delay before sacrifice. Both drugs increase tryptophan, 5-HIAA and 5-HT concentrations in the brain. U-14,624 has been studied by Johnson *et al.*[7,8] and fusaric acid has been shown to increase brain 5-HT:[9] our results are in agreement with these studies.

The tryptophan levels in the blood, following administration of fusaric acid and U-14,624, however, show some interesting differences. Fusaric acid increases the blood-free tryptophan concentration probably by displacing tryptophan from its protein bindings in plasma. This hypothesis is supported by the drop of total tryptophan, as a consequence of an increased brain 5-HT synthesis, and the similarity of the structure of the inhibitor to that of tryptophan: the carboxylic function and a pyridine ring moiety of the indole nucleus, might be valuable explanations for this phenomenon. Acetylsalicylic acid also increases the blood-free tryptophan and a similar hypothesis has been suggested.[10]

The large increase in blood-free tryptophan leads to a facilitated uptake of tryptophan by the brain tissue and thus to an increased synthesis of 5-HT, confirmed by the augmentation of 5-HIAA. It is also possible that, as an inhibitor of DBH, fusaric acid may act like the other compounds having the same property: for these compounds it is possible that the increase in serotonin synthesis could be directly related to the NE depletion.

TABLE 1. EFFECT OF U-14,624 AND FUSARIC ACID TRYPTOPHAN METABOLISM IN BLOOD AND BRAIN

Inhibitor (100 mg/kg i.p. for 3 hr)	Blood		Brain				
	Total TRY (% of control)	Free TRY (% of control)	TRY (% of control)	5-HT (% of control)	5-HIAA (% of control)	NE (% of control)	DA (% of control)
U-14,624	124†	88	130‡	126‡	130‡	39‡	121
Fusaric acid	80*	198‡	181‡	117†	152‡	56‡	113

* P 0·05.
† P 0·01.
‡ P 0·001.

We can conclude, so far, that fusaric acid acts in two ways on the metabolism of 5-HT: on one hand, a central effect common to most DBH inhibitors and, on the other hand, a peripheral action due to the inhibition of the binding of tryptophan to serum albumin. Studies are in progress to confirm this hypothesis and to try to dissociate the two components of the fusaric acid action.

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Effects of a water-soluble carbodiimide on the osmotic fragility and ion permeability of erythrocytes

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GODIN and Schrier[1] have shown that a progressive inactivation of ATPase activity was found when human erythrocyte ghosts are treated with the water-soluble carbodiimide, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC), with the Mg^{2+}-dependent ATPase showing a greater susceptibility to inactivation when compared with the Na^{+}-K^{+}-Mg^{2+}-dependent ATPase. These authors conclude that carbodiimide attack on the erythrocyte membrane causes a selective structural perturbation which in turn causes a disruption of the ATP-hydrolysing system. We have previously suggested[2–7] that the Mg-ATPase located in the erythrocyte membrane could be implicated in the control of passive ion permeability and of the disc shape of this cell. Schoffeniels[8] has extended this hypothesis and regards the Ca^{2+}-stimulated Mg^{2+}-dependent ATPase as being of particular importance in the control of cation permeability. The purpose of the experiments reported in this communication was to determine whether EDAC applied to the outer surfaces of intact pig erythrocytes was able to modify their anion permeability and osmotic fragility.

Heparinized pig blood was obtained fresh from the slaughterhouse, centrifuged and the plasma and buffy coat discarded. Erythrocytes were washed three times in 0·9% NaCl (pH 7·4), and the final PCV was suspended in an equal volume of 0·9% NaCl. Three ml of suspended erythrocytes were mixed with 1 ml 0·9% NaCl which contained EDAC. The concentrations of EDAC given are the final concentration of this agent during the period of preincubation. Exposure to EDAC was for varying periods, but was usually for 30 min, either at 19° or on ice. 0·1 ml samples were then taken and added to 5 ml NaCl solution of appropriate dilution. The method of determination of osmotic resistance (fragility) followed that of Parpart *et al.*;[9] NaCl solutions (including those described above) were made by dilution of a stock NaCl–PO_4 solution to give a standard pH of 7·40. Erythrocytes and NaCl were equilibrated for 45 min in a water bath at 25° and haemolysis was stopped by the addition of the complementary solution[9] which served to return the osmotic pressure around the unhaemolysed cells to normal tonicity. Erythrocytes were then removed by centrifugation and the supernatant was read at 540 nm.