TABLE 2. DIFFERENCES IN ATP, CREATINE PHOSPHATE, AND LACTATE BETWEEN ZERO TIME AND 15 SECONDS*

	ATP phosphate	Creatine	Lactate	$\Delta \geqslant P$
Control	0·51	2·79	2·70	6·00
Phenobarbital	0·44	2·84	1·85	5·13
GHB	0·89	1·80	2·29	4·98

* The sum of these differences may be taken as a measure of the production and utilization of high-energy ($\geqslant$) phosphate during this period.

of drug/kg, as compared to controls. Thus, studies *in vitro* did not clarify events observed *in vivo*.

By adding the differences in ATP, creatine phosphate, and lactate between zero time and 15 sec, a measure of production and utilization of high-energy phosphate over this period of ischemia may be obtained (Table 2). This amount was very close in the phenobarbital and GHB animals. Krebs cycle substrate concentrations were very similar as well. The anesthetized animals differed from controls in their diminished malate concentration at zero time with a rise after 15 sec of ischemia. Similar results have been noted by Goldberg *et al.*[7] after treatment with a variety of anesthetics.

Although the rate of glycolysis in brain during 15 sec of ischemia was depressed as much in the GHB mice as in those given phenobarbital, the appearance of the animals differed considerably. The animals on phenobarbital were deeply depressed, limp, and flaccid. The animals after GHB were more lightly anesthetized at the dosage chosen and some retained a righting response. They had a marked exophthalmos and showed occasional brief, bilaterally synchronous myoclonic twitching. Thus, although the magnitude of fall in overall cerebral metabolic activity was similar in the two groups it was concluded that GHB affects to a greater or lesser extent discrete and different portions of brain compared to those affected by phenobarbital, or affects differently some metabolic site not examined in the present investigation.

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Decarboxylase inhibitors and histamine in guinea pigs*

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THE experiments reported here were performed to study several histidine decarboxylase inhibitors for their effects upon tissue histamine concentration and anaphylaxis in guinea pigs.

METHODS

Young guinea pigs of both sexes were used. In an effort to deplete the animals of any easily releasable histamine they were first subjected to passive anaphylactic shock with a supralethal amount

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of antiserum, but protected from death with diphenhydramine hydrochloride. The survivors were selected for treatment or control groups by the flip of a coin. The compounds which were used were then given intraperitoneally. Control animals were given 0·9% saline in a volume similar to the experimental drug being studied. At the end of the drug trial (usually 4 weeks) the animals were again subjected to passive anaphylaxis. Those animals which did not succumb within 15 mins were killed by a blow on the head. The major blood vessels of the neck were severed immediately. Tissues were removed from the animals, blotted free of blood, weighed, and homogenized in 5% trichloroacetic acid within a few minutes. The extracts were assayed for histamine.[1]

Drugs.† NSD 1024 and NSD 1034 (Fig. 1) were gifts from D. J. Drain of Smith & Nephew Research, Ltd., Gilson Park, Harlow-Essex, England. Ten per cent solutions in saline were prepared. Six animals received NSD 1024, 70 mg/kg twice daily, and six received NSD 1034, 70 mg/kg twice daily. Methylprednisolone acetate was given intramuscularly in the anterior thigh at weekly intervals. Seven animals received 56 mg/kg each week for 4 weeks. Two animals which then survived an anaphylactic challenge were continued on the same weekly dose for an additional 15 weeks. α-Methyl-5-hydroxytryptophan (α-me5HTP) was a gift from Carl Schlagel, Ph.D., of the Upjohn Co., Kalamazoo, Mich. Solutions of 0·5% in saline were administered i.p. in amounts of 0·1 g/kg twice daily for 1·5 days to four animals, for 3 days to three animals and for 8 days to eight animals. R04-4602 (Fig. 1) was a gift from Dr. A. Pletscher, Medical Research Department, F. Hoffman-LaRoche & Co., Basle, Switzerland. A solution of 20% R04-4602 in saline was injected into 18 guinea pigs i.p. in amounts varying from 0·1 to 1·0 g/kg for 1 to 20 days. Pyrocatechol in saline was prepared in 0·5% solution. Twenty mg/kg was injected i.p. twice daily to five animals. Three survived for 31 days. Semicarbazide was dissolved in saline to make a 2% solution. Twenty mg/kg was injected i.p. twice daily to five animals; four survived for 31 days. 2-Thiolhistidine was a gift from A. Pletscher, Hoffman LaRoche & Co. Seven mg of a 0·05% solution in saline was injected per kg i.p. twice daily to five animals for 22 days.

RESULTS AND DISCUSSION

Tissue concentrations of histamine. The mean histamine concentrations of the tissues removed from guinea pigs are given in Table 1 where they are expressed as micrograms histamine per gram (wet weight) of tissue. Because the various control groups were not significantly different from one another in the amount of histamine present, those results were pooled for comparison with each of the treatment groups. Two animals that died of anaphylaxis but were not treated with saline were included with the controls. Mean values which were significantly different statistically from control means on the basis of Student's '*t*' test are indicated in the table. The most striking differences were the lower mean lung histamine content in animals treated with NSD 1034, methylprednisolone, R04-4602, and pyrocatechol. Mean stomach histamine content was significantly lower than controls in the methylprednisolone- and semicarbazide-treated animals and higher in the NSD 1024-treated animals. Several other apparently significant differences between treatment and control group means fell short of statistical significance when a correction was introduced for significant differences between the standard errors of the means which were compared.

The mean weights of some of the groups of treated animals were significantly different from the mean weights of the controls. Since the histamine content of several organs varies with body weight,† analyses of covariance were used to adjust the mean tissue histamine concentrations for the groups with body weight significantly different from the controls. The adjusted means in Table 2 show that the differences from control groups of the lung histamine concentration in NSD 1034- and pyrocatechol-treated animals and the stomach histamine concentration in methylprednisolone-treated animals could have been due to differences in body weight alone.

NSD 1024 and NSD 1034 are powerful inhibitors *in vitro* of both the specific histidine decarboxylase of rat hepatoma (FhepHD) and the amino acid decarboxylase of guinea pig kidney (GPHD).[2] The amounts of these agents given in this study should have been sufficient to inhibit both enzymes unless they were somehow inaccessible to compounds in the blood. Absorption of both NSD 1024 and NSD 1034 from the peritoneum was confirmed by the appearance of the unaltered drug in paper

* Drug doses and histamine values are given in terms of the base.

† Unpublished results.

TABLE 1. TISSUE HISTAMINE CONCENTRATIONS AFTER VARIOUS DRUGS*

Group	No.	Wt.	Lung	Heart	Liver	Kidney	Stomach	Jejunum	Abdominal skin-
Controls	35	333·4 ± 11·7	30·3 ± 1·8	8·1 ± 0·4	2·6 ± 0·2	3·9 ± 0·3	9·7 ± 0·5	17·3 ± 0·7	5·1 ± 0·4
NSD 1024	4	432·5 ± 28·1	33·2 ± 10·7	10·2 ± 1·8	2·3 ± 0·6	6·5 ± 1·8‡	13·4 ± 2·3‡	29·4 ± 5·9‡	5·2 ± 0·5
NSD 1034	6	413·0 ± 27·2†	19·4 ± 3·2†	8·7 ± 0·6	2·4 ± 0·6	4·2 ± 1·1	10·8 ± 2·4	16·6 ± 2·4	4·6 ± 0·4
Methylprednisolone	8	418·5 ± 42·6‡	13·6 ± 2·3†	5·5 ± 1·1‡	1·9 ± 0·4	3·5 ± 1·6	6·0 ± 1·1†	16·1 ± 1·8	3·7 ± 0·7
α-Me5HTP	15	370·3 ± 9·6	25·0 ± 2·4	7·6 ± 0·9	2·6 ± 0·4	3·7 ± 0·6	10·0 ± 0·7	19·7 ± 1·5	3·9 ± 0·4
R04-4602	17	333·8 ± 20·1	19·4 ± 2·6†	7·1 ± 0·5	2·3 ± 0·2	3·2 ± 0·3	8·4 ± 0·9	17·2 ± 1·6	4·2 ± 0·3
Pyrocatechol	3	449·7 ± 9·6†	15·5 ± 3·9†	5·1 ± 1·4‡	1·3 ± 0·5‡	3·9 ± 1·8	9·1 ± 1·1	15·8 ± 2·9	4·9 ± 0·8
Semicarbazide	4	333·3 ± 20·2	18·8 ± 4·2	5·7 ± 0·5	2·1 ± 0·2	3·0 ± 0·5	6·1 ± 0·1†	16·9 ± 1·5	4·0 ± 0·4
2-Thiolhistidine	5	410·0 ± 16·3†	34·6 ± 1·9	6·2 ± 1·9	2·5 ± 0·6	2·9 ± 0·6	12·2 ± 2·5	21·9 ± 2·2‡	4·7 ± 0·6

* Mean ± standard error.
† Statistically significant: $P < 0.05$ (Student's t test).
‡ Statistically significant by t test, but not so when computed with a correction for the differences in the standard errors of the two means.

TABLE 2. HISTAMINE CONCENTRATION*

Group	Lung	Heart	Liver	Kidney	Stomach	Jejunum	Skin
Controls	30·3 ± 1·8	8·1 ± 0·4	2·6 ± 0·2	3·9 ± 0·3	9·7 ± 0·5	17·3 ± 0·7	5·1 ± 0·4
NSD 1024	36·1 ± 5·5	10·3 ± 1·4	2·2 ± 0·6	5·8 ± 1·2	13·3 ± 1·8	28·5 ± 2·8‡	5·9 ± 0·8
NSD 1034	21·5 ± 4·5	8·8 ± 1·1	2·3 ± 0·5	3·7 ± 0·9	10·7 ± 1·5	16·0 ± 2·3	5·1 ± 0·8
Methylprednisolone	14·7 ± 4·8†	6·3 ± 1·2	1·7 ± 0·5	4·3 ± 1·0	6·4 ± 1·6	15·4 ± 2·4	3·5 ± 0·7
Pyrocatechol	19·3 ± 6·4	5·3 ± 1·6	1·1 ± 0·7‡	3·1 ± 1·3	8·9 ± 2·1	14·7 ± 3·2	5·8 ± 1·0
2-Thiolhistidine	36·6 ± 4·9	6·3 ± 1·2	2·4 ± 0·5	2·5 ± 1·0	12·1 ± 1·6	21·3 ± 2·5	5·2 ± 0·7

* In micrograms per gram; means ± standard error, adjusted for the effect of body weight.
† Statistically significant (S.E.D. > 2·0).
‡ See Table 1.

chromatograms of the urine. Nevertheless, long-continued treatment had no effect on tissue histamine content.

α-Me5HTP and pyrocatechol are potent inhibitors of GPHD but are not particularly effective against FHepHD.[3, 4] Their failure to influence histamine concentration may have been due to their inactivity against FHepHD.

R04-4602 has been shown to be a potent decarboxylase inhibitor,[5, 6] but no specific studies of its effects on histidine decarboxylase have been reported. Treated animals did have a small but significant decrease in mean lung histamine content. Pulmonary toxicity prevented the use of larger doses.

Adrenal corticosteroids in amounts similar to the smaller of the two dosages of methylprednisolone used in these experiments have produced marked decreases in histamine content and histidine decarboxylase activity of rat tissues.[7, 8] The changes induced in guinea pigs, however, were quite small.

Histamine incorporated into tissues as a result of histidine decarboxylation has an extremely slow turnover.[9] This would certainly make it difficult to alter histamine content by inhibition of histamine formation. For this reason all the experimental drugs except α-me5HTP were given for approximately a month. It is possible that even this length of time was inadequate to deplete tissue histamine stores. Depletion of preformed tissue histamine by preliminary anaphylaxis was attempted as a means of augmenting the response to enzymatic inhibitors, but this did not seem to be helpful.

It is clear that the histamine present in guinea pig tissues is not easily depleted by any means. Since partial depletion does not alter susceptibility to anaphylaxis this approach to the prevention of harmful effects from antigen–antibody reactions seems to be a particularly difficult one.

None of the drugs described protected guinea pigs from anaphylaxis. This failure to protect would be expected with the groups whose tissue histamine levels did not change but was a little surprising in those treatment groups, methylprednisolone and R04-4602, which showed slight but significant decreases in histamine content.

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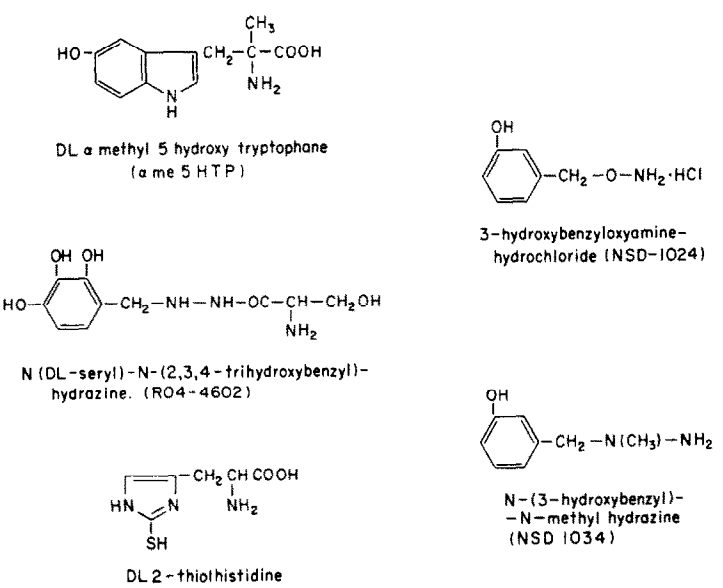

FIG. 1. Structures of several of the compounds used in this study.

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Lack of metaraminol biotransformation by rabbit tissues *in vitro*

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METARAMINOL (1-*m*-hydroxy norephedrine) has been shown to possess significant clinical utility in the treatment of acute hypotension of diverse etiolgy.[1, 2] In addition to its well-established usefulness as a therapeutic agent, metaraminol has recently received widespread attention as an experimental tool in autonomic pharmacology as a result of its ability to deplete tissue catecholamine stores.[3, 4] Although it has been demonstrated that small amounts of unchanged metaraminol remain bound in tissues for one to two weeks after injection[4, 5] no reports have appeared on the biotransformation of the drug *in vivo* or *in vitro*. The present comunication describes studies of the metabolism of metaraminol *in vitro*.

Tissues were removed from adult male New Zealand rabbits and homogenized at 0°–5° in a Potter-Elvehjem-type homogenizer. Hepatic mitochondria, as a source of monoamine oxidase (MAO), were prepared from liver homogenates by the procedure of Schneider.[6] Amphetamine deaminase, an enzyme system known to oxidatively deaminate several sympathomimetic amines possessing α-methyl groups, was prepared from liver homogenates according to Axelrod.[7] Hepatic microsomal glucuronyl transferase was prepared by the method of Hsia *et al.*[8] All incubations were carried out in a Dubnoff metabolic shaking apparatus at 37° under air.

In MAO experiments, the incubation mixture was that described by Zile and Lardy.[9] Table 1 presents data showing that, although tyramine was metabolized at a brisk rate by MAO, no disappearance of metaraminol was observed. To examine the possibility that MAO action on metaraminol might produce a metabolite with extraction characteristics and extinction properties similar to those of metaraminol, paper chromatography was utilized. Extracts of incubation mixtures were spotted on strips of Whatman 1 paper and developed by the ascending technic for 19 hr in three solvent systems: (1) n-butanol: acetic acid:water (12:3:5); (2) isopropanol:ammonia:water (8:1:1); (3) isobutanol saturated with 0·1 N HCl. The chromatograms were air dried, and spots were visualized by spraying with Gibbs reagent followed by borate buffer (pH 9·2). In all three systems single spots were observed having the same R_f values as authentic metaraminol, i.e. 0·66, 0·81, and 0·42 respectively.

Axelrod[7] showed amphetamine deaminase to be capable of deaminating several phenyl isopropyl amine derivatives. Since metaraminol is a doubly hydroxylated phenyl isopropyl amine, it was reasoned that it might serve as a substrate for the enzyme. Table 2 presents data demonstrating that no metabolism of metaraminol occurred in the presence of amphetamine deaminase. Experiments in which the amounts of enzyme and cofactors present in the incubation medium were increased by 3 to 4-fold demonstrated that, although amphetamine metabolism was enhanced by about 4 to 5-fold no disappearance of metaraminol could be detected.