the reductions after sympathectomy and after reserpine treatment are completely independent. Thus it is more likely that COMT exists both intraneuronally and extraneuronally and that its depression after reserpine is not mediated by substrate depletion (Marsden & others, 1971).

There are indications for the existence of two forms of COMT in the rat salivary gland, one form appears to be connected with the atrophied gland and the other with the sympathectomized one (Broch, unpublished).

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REFERENCES

BROCH, O. J. (1973). *Acta pharmac. tox.*, **33**, 417–428.
MARSDEN, C. A., BROCH, O. J. & GULDBERG, H. C. (1971). *Eur. J. Pharmac.*, **15**, 335–342.

Inhibition of a minor pathway of L-dopa metabolism in the intestinal lumen using a decarboxylase inhibitor (Ro 4-4602)

The evidence for the existence of a minor pathway of L-dopa metabolism through the action of the intestinal microflora has been reviewed recently (Bakke, 1973). After the introduction of decarboxylase inhibitors in the treatment of parkinsonism to reduce the extracerebral conversion of orally administered L-dopa to dopamine, the question has arisen whether the metabolism in the intestinal lumen is also affected by such inhibitors. We have carried out experiments with cultures of intestinal microorganisms and with rats to study the effect of a decarboxylase inhibitor Ro 4-4602 (Benserazid, F. Hoffman-La Roche & Co., Switzerland) on the microbial metabolism of L-dopa and some related phenolic acids.

The methods used to study the metabolism in anaerobic cultures of mixed caecal microorganisms have been described previously (Bakke, 1971). The substrate concentration was 0·5 mg ml^{-1} and aliquots of the incubates contained from 10 μg to 2 mg ml^{-1} of the decarboxylase inhibitor.

After anaerobic incubation of L-dopa with caecal microorganisms at 37° for 44 h, the formation of 3,4-dihydroxyphenylacetic acid, 3-hydroxyphenylacetic acid, 3-hydroxyphenylpropionic acid and 4-methylcatechol was demonstrated by t.l.c. None of these metabolites was found in the incubates containing Ro 4-4602 at a concentration of 1 mg ml^{-1} and partial inhibition was obvious with concentrations down to 0·1 mg ml^{-1}. These findings suggested that several steps in the microbial pathway could be inhibited by Ro 4-4602.

When 3,4-dihydroxyphenylacetic acid was used as a substrate, the amount of the *para* dehydroxylated metabolite 3-hydroxyphenylacetic acid was significantly reduced in the presence of 0·1 mg ml^{-1} of the inhibitor. Higher concentrations were required to affect decarboxylation to 4-methylcatechol.

The need for higher concentrations in order to inhibit microbial decarboxylation was also demonstrated using 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid and 3,4-dihydroxycinnamic acid as substrates. The higher potency of Ro 4-4602 as an inhibitor of dehydroxylation was clearly demonstrated with 3,4-dihydroxycinnamic acid. This compound is metabolized by intestinal microorganisms by *para* dehydroxylation and reduction to 3-hydroxyphenylpropionic acid and by decarboxylation and reduction to 4-vinylcatechol and 4-ethylcatechol (Scheline, 1968). The formation of the deyhdroxylated metabolite was abolished in the presence of 1 mg ml^{-1} of Ro 4-4602 whereas the amounts of the decarboxylated metabolites 4-vinylcatechol and 4-ethylcatechol equalled or exceeded the quantities produced in the absence of the inhibitor. The latter finding is probably due to larger amounts of substrate becoming available to the decarboxylases after inhibition of dehydroxylation.

In view of the findings *in vitro*, the amount of a dehydroxylated metabolite of L-dopa, 3-hydroxyphenylacetic acid, in rat urine was measured to assess inhibition of the intestinal microflora by Ro 4-4602 *in vivo*. 3-Hydroxyphenylacetic acid arises from L-dopa exclusively through the action of the microflora (Borud, Midtveld & Gjessing, 1973; Goldin, Peppercorn & Goldman, 1973).

A group of 5 male rats (225–245 g) was fed a commercial pellet diet with 0·4% w/w of L-dopa for 4 days. Then the animals were changed to the pellet diet containing no added L-dopa and each rat received 60 mg of L-dopa by stomach tube. The individual urines were collected for the following 24 h. A second group of rats received the L-dopa containing diet with 0·1% w/w of Ro 4-4602 for 4 days and then 60 mg of L-dopa and 15 mg of the inhibitor by stomach tube before urine collection. A pretreatment period was used to obtain steady state and identical intake of L-dopa in the two experimental groups was assured by giving the last dose by stomach tube. This dose matched the mean individual 24 h intake of the drugs during the pretreatment period.

Aliquots of the individual urines were acidified with HCl and the phenolic fraction was extracted with ethyl acetate. The trimethylsilyl derivatives were prepared and separated by g.l.c. using a 3% OV-25 column (¼ in. × 5 ft) at 140° with argon (35 ml min^{-1}) as the carrier gas. The 24 h excretion of 3-hydroxyphenylacetic acid was calculated from the peak areas by comparison to chromatograms obtained with the same urine after adding known amounts of the authentic metabolite.

The amount of 3-hydroxyphenylacetic acid found in the urine from the rats given L-dopa alone was 1·8 ± 0·3 mg 24 h^{-1} (mean ± s.e.). The corresponding value in the group given both L-dopa and Ro 4-4602 was 0·8 ± 0·2 mg 24 h^{-1}. The difference is statistically significant ($P < 0·02$).

In summary, we have shown that the peripheral decarboxylase inhibitor Ro 4-4602 inhibits bacterial metabolism of L-dopa and related phenolic acids *in vitro*. However, with the catechol compounds studied, Ro 4-4602 is more potent as an inhibitor of *para* dehydroxylation than of microbial decarboxylation. The significant reduction in the excretion of 3-hydroxyphenylacetic acid when Ro 4-4602 is given to rats treated with L-dopa suggests that the microbial pathway in the intestine is affected by this inhibitor also *in vivo*.

We have now carried out identical *in vitro* experiments with carbidopa (MK 486, Merck, Sharp & Dohme), an alternative peripheral decarboxylase inhibitor used together with L-dopa. The formation of 3-hydroxyphenylpropionic acid and 4-methylcatechol from L-dopa in the incubates was not affected by this inhibitor (0·1–1·0 mg ml^{-1}). The amount of 3,4-dihydroxyphenylacetic acid arising from L-dopa was increased and the formation of 3-hydroxyphenylacetic acid was reduced in the presence of carbidopa. Thus, carbidopa has no appreciable effect on the microbial degradation of L-dopa *in vitro* other than an inhibitory effect on a late step involving *para*-dehydroxylation of 3,4-dihydroxyphenylacetic acid.

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REFERENCES

BAKKE, O. M. (1971). *Acta pharmac. tox.*, **30**, 115–121.

BAKKE, O. M. (1973). In *Advances in Neurology*, vol. 3, *Progress in the Treatment of Parkinsonism*, 189–197. Editor: D. B. Calne. New York: Raven Press.

BORUD, O., MIDTVEDT, T. & GJESSING, L. R. (1973). *Acta pharmac. tox.*, **33**, 308–316.

GOLDIN, B. R., PEPPERCORN, M. A. & GOLDMAN, P. (1973). *J. Pharmac. exp. Ther.*, **186**, 160–166.

SCHELINE, R. R. (1968). *Acta pharmac. tox.*, **26**, 189–205.

Imidazole and penicilloylation in penicillin allergy

Recently Bundgaard (1972) discussed some aspects of an imidazole-catalysed penicilloylation and suggested that *N*-penicilloyl imidazole is a reactive intermediate capable of transferring penicilloyl groups to acceptor amino-groups and other nucleophiles. This reaction is thought to be of potential significance in penicillin allergy since it could be involved in the efficient formation of penicilloyl antigenic determinants *in vivo*. Direct penicilloylation of primary amino-groups is quite slow in neutral aqueous solution and its significance as an antigen forming step *in vivo* is therefore not self-evident. For this reason we had studied (Schneider & de Weck, 1968, 1969) the direct neutral penicilloylation of a number of functional groups occurring on proteins hoping to find (inter alia) accelerated penicilloylations. In these investigations, the penamaldate stability test (Schneider & de Weck, 1966), which enables the formation of stable penicilloyl derivatives in the presence of penicilloic acid to be detected, played a key role. With regard to the imidazole function, our screening program showed that no penicilloyl derivatives detectable by the penamaldate technique would form after incubation of benzylpenicillin with either N^{α}-Z-DL-histidine or N^{α}-Z-L-histidylglycine at pH 9 and 10·5 respectively. On the other hand these incubations revealed a catalysed hydrolysis of penicillin to penicilloic acid which was also reported.

These results are only in apparent conflict with the report of Bundgaard (1972) on the formation of unstable and intermediary penicilloyl imidazoles. In order to clarify this point we present here some additional data on penicilloylation and penicilloic acid formation in the presence of imidazole compounds which were either not included in the communications of 1968 and 1969 or have accumulated since.

With the incubation and measurement technique described in detail before (Schneider & de Weck, 1968), we found at pH 8·5 that 0·36 M imidazole accelerates