The Effect of Monoamine Oxidase Inhibitors on Conjugation

A number of monoamine oxidase inhibitors have been reported to cause jaundice. These include iproniazid, isocarboxazid, nialamide and phenelzine[1]. The jaundice is similar to that seen in viral hepatitis but the mortality is much higher. In animals the drugs do not cause hepatic lesions unless given in large amounts[2]. Since the cause of the jaundice is not known the effect of various monoamine oxidase inhibitors on conjugation has been studied in vitro.

The rate of bilirubin glucuronide formation was determined in rat liver slices and rabbit liver homogenates by the method of LATHE and WALKER[3]. *o*-Aminophenol conjugation was determined in rat liver slices by the method of LEVVY and STOREY[4]; in rabbit liver homogenates by the method of STEVENSON and DUTTON[5]. Isonicotinic acid hydrazide, iproniazid, phenelzine, nialamide and isocarboxazid were added to give final concentrations of 0.01, 0.1, 1.0 and 10.0 m*M*.

The addition of phenelzine and isocarboxazid lowered the rate of conjugation of bilirubin by rat liver slices, whereas iproniazid and nialamide increased the rate of conjugation. Isonicotinic acid hydrazide had no effect on bilirubin conjugation by rat liver slices (Table I). Conjugation of bilirubin by rabbit liver homogenates was inhibited by iproniazid, phenelzine, nialamide and isocarboxazid (Table II). Isonicotinic acid hydrazide did not alter the rate of conjugation. All the drugs tested inhibited conjugation of *o*-aminophenol in rat liver slices (Table I) and in rabbit liver homogenates (Table II).

The effect of these drugs on conjugation was investigated to try and determine the site of action of the monoamine oxidase inhibitors causing jaundice. The effect of the substances tested on conjugation in vitro is variable depending on the substrate used. *o*-Aminophenol conjugation was inhibited in liver slices and homogenates. The conjugation of bilirubin is altered depending on whether liver slices or homogenates are used.

In liver slices interference with conjugation can occur at a number of sites. The aglycone must penetrate the liver cell membrane, it is then conjugated with glucuronate and the conjugate excreted from the liver cell through the surrounding membrane. Interference with the passage of the aglycone or conjugate at these points would decrease the conjugate formed. In rabbit liver homogenates the transferase (UDP glucuronate glucuronyl transferase [acceptor unspecific] E.C. 2.4.1.17.) stage of conjugation is examined. In this preparation the endoplasmic reticulum of the liver cell containing the enzyme is exposed to the substrate and the drug although the enzyme is probably surrounded by a lipid membrane. For conjugation to occur the substrate must pass through the lipid membrane to the enzyme and the conjugate must pass through the membrane from the enzyme to the surrounding medium. Inhibition of conjugation in homogenate or microsome preparations could occur at the entry to the

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Table I. Effect of monoamine oxidase inhibitors and similar drugs on bilirubin and *o*-aminophenol conjugation in rat liver slices

Concentration (m*M*)		Bilirubin conjugation					*o*-Aminophenol conjugation			
		0.01	0.1	1.0	10.0		0.01	0.1	1.0	10.0
Isonicotinic acid hydrazide	(0.050)	0	0	0	0	(0.29)	0	0	0	− 95
Iproniazid	(0.056)	0	0	+ 28	+ 125	(0.28)	+ 20	+ 20	+ 49	− 37
Phenelzine	(0.036)	0	− 29	− 35	− 38	(0.30)	0	− 20	− 74	− 83
Nialamide	(0.044)	+ 18	+ 32	+ 76	+ 99	(0.31)	0	0	0	− 94
Isocarboxazid	(0.057)	− 30	− 15	− 10	− 20	(0.30)	− 20	− 20	− 26	− 83

Conjugation in controls in parentheses (mμ moles/mg wet weight/h). Results are expressed as % difference from control. Each result is the mean of 3 experiments.

Table II. Effect of monoamine oxidase inhibitors and similar drugs on bilirubin and *o*-aminophenol conjugation in rabbit liver homogenates

Concentration (m*M*)		Bilirubin conjugation					*o*-Aminophenol conjugation			
		0.01	0.1	1.0	10.0		0.01	0.1	1.0	10.0
Isonicotinic acid hydrazide	(0.47)	0	0	0	0	(6.3)	+ 24	+ 13	− 28	− 100
Iproniazid	(0.35)	0	0	− 12	− 44	(5.8)	0	0	− 8	− 83
Phenelzine	(0.52)	0	− 13	− 54	− 83	(7.2)	0	0	− 20	− 27
Nialamide	(0.40)	− 20	− 27	− 20	− 100	(6.3)	0	0	− 40	− 100
Isocarboxazid	(0.46)	0	− 17	− 25	− 100	(6.7)	0	− 10	− 20	− 52

Conjugation in controls in parentheses (mμ moles/mg wet weight/h). Results are expressed as % difference from control. Each result is the mean of 3 experiments.

microsome, at the enzyme or at the exit from the microsome.

Isonicotinic acid does not alter the rate of bilirubin formation. Phenelzine and isocarboxazid decrease the rate of conjugation of bilirubin in slices and the effect could be explained by enzyme inhibition. The stimulation of bilirubin conjugation in slices by iproniazid and nialamide and the inhibition of conjugation in homogenates is more difficult to explain. It may be that the drug facilitates the passage of bilirubin through the slice to the enzyme site, a process which it cannot do in homogenates. An alternative explanation is that since the relationship between enzyme inhibition and stimulation is so close, conditions in the slice may facilitate enzyme stimulation rather than enzyme inhibition.

The experiments show that the tranferase conjugating *o*-aminophenol is usually inhibited whereas the effect of the drugs on bilirubin conjugation is variable. Since the liver slice more closely resembles the intact liver rather than the homogenate preparations the cause of monoamine oxidase inhibitor jaundice cannot be explained on inhibition of bilirubin glucuronyl transferase alone. The process causing the jaundice is probably complex and it may be that formation of hepatotoxic intermediaries by metabolism of the drug in the endoplasmic reticulum may play a part as well as possible alterations in cell permeability and an effect on the enzyme[6].

Zusammenfassung. Die Wirkung von Monoamino-Oxidase-Blockern in Verbindungen ist in vitro veränderlich und hängt vom benutzten Substrat und Gewebe ab. Im allgemeinen wird die *o*-Aminophenol-Verbindung blockiert, während die Bilirubinverbindung verhindert oder auch angeregt werden kann. Die verschiedenen Wirkungen können von Variationen von der Verfügbarkeit des Substrats für die Enzyme abhängen. Es ist sehr unwahrscheinlich, dass die Monoamino-Oxidase-Blocker-Gelbsucht aus der Blockierung der Glucuronyl-Transferase allein zu erklären ist.

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Norepinephrine Depleting and Antihypertensive Effect of 4-Methoxy-3,5-dihydroxyphenylalanine

Amongst several amino acids studied as potential metabolic precursors of false adrenergic transmitters, 5-hydroxydopa (5-HO-dopa) was found to induce a marked norepinephrine depletion in peripheral sympathetically innervated organs of various species[1]. Adrenergic transmission in cats pretreated with 5-HO-dopa was greatly impaired[1,2]. 5-Hydroxydopamine and its O-methylated and/or β-hydroxylated metabolites were found to be stored in adrenergic nerves and liberated as false adrenergic transmitter substances[1-3].

In the course of further studies the para-O-methylated derivative of 5-HO-dopa proved to be an even more potent depletor and showed interesting antihypertensive properties in renal hypertensive rats.

The norepinephrine depleting action of 4-methoxy-3,5-dihydroxyphenylalanine was studied in rats which were pretreated with either 100, 50, 25 or 12.5 mg/kg 20 and 4 h before sacrifice. The compound was given as aqueous suspension by stomach tube. The norepinephrine content of heart and brain was determined according to the method of BERTLER et al.[4]. The norepinephrine values are expressed in % of control values obtained in rats which were given saline instead of the drug.

As shown in the Table, there is a clear-cut dissociation between the norepinephrine depleting effect in brain and heart. The dissociation seems to be somewhat smaller than in the case of 5-HO-dopa. However, at the doses used in these experiments the animals showed no noticeable changes in gross behaviour, especially no sedation.

In a second series of experiments the effect of pretreatment with 4-methoxy-3,5-dihydroxyphenylalanine on the blood pressure of renal hypertensive rats was studied. Systolic blood pressure was measured according to the method described by GEROLD et al.[5]. After 3 control days the rats were given 100 mg/kg twice daily for $2^1/_2$ days (a total of 5 doses) either by stomach tube or i.p. As shown in the Figure, 4-methoxy-3,5-dihydroxyphenylalanine markedly reduced the elevated blood pressure both after oral and i.p. administration.

In experiments with isolated perfused spleens of pretreated cats the norepinephrine output and the contractile response to sympathetic nerve stimulation were greatly reduced and 4-methoxy-3,5-dihydroxyphenethylamine was liberated as a false sympathetic transmitter. The detailed results of these experiments will be published elsewhere.

The effect of 4-methoxy-3,5-dihydroxyphenylalanine on the norepinephrine content of heart and brain of the rat

Doses of 4-methoxy-3,5-dihydroxyphenylalanine[a]	% Controls	
	Heart	Brain
2 × 100 mg/kg	2.8 ± 1.4	26.6 ± 2.2
2 × 50 mg/kg	13.5 ± 2.1	36.2 ± 2.5
2 × 25 mg/kg	45.7 ± 7.8	67.0 ± 3.0
2 × 12.5 mg/kg	55.8 ± 7.5	80.5 ± 4.2

[a] The single doses were given by stomach tube 20 and 4 h before sacrifice.

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