panied by increased pulmonary arterial pressure[15]. Thus, the concomitant reduction of pulmonary pressure and resistance elicited by hexoprenaline in the present study was probably due to active vasodilation rather than to passive, flow-induced distension of the hypertensive vessels. The excessive vasodilatory response to hexoprenaline suggested that the sustained vascular resistance during acute normoxia was due partly to persistent smooth muscle tone rather than solely to morphological thickening of the vessel wall. Although this concept has been stated[8,9], there is little data to support it, and it is not widely appreciated. The concept is compatible with a study of a calf with pulmonary hypertension in which 24 h of alveolar normoxia reduced pulmonary arterial pressure considerably more than did 10 min of oxygen breathing[18].

If it could be shown that the response to acute normoxia was not indicative of the total potential for relaxation of the hypertensive pulmonary vessels, then a secondary aim of this study was to gain some insight into the cause of the sustained smooth muscle tone. Free cytoplasmic calcium is a determinant of smooth muscle tone[19], and increased levels of calcium have been observed in the pulmonary smooth muscle of chronically hypoxic animals[20]. Thus, pharmacologic agents that alter the concentration of activator calcium were used as pulmonary vasodilators. Verapamil was chosen because it reduces transmembrane calcium influx that accompanies membrane depolarization[10,11], does not have adrenergic activity or act as a competitive inhibitor of any humoral mediator[12,13] and blocks the pulmonary pressor response to acute alveolar hypoxia[21,22]. The vasodilatory response to verapamil in the present study suggests that the transmembrane influx of extracellular calcium plays a role in the mechanism of chronic, hypoxia-induced pulmonary hypertension. Hexoprenaline was used because it is a β-adrenergic agonist that acts on β-2 (vasodilatory) type receptors[14,15], the spasmolytic effect of β-adrenergic activation is possibly related to both enhanced cellular sequestration and extrusion of free calcium[19,23,24] and reduction of calcium influx via hyperpolarization of the plasma membrane[24], and orciprenaline, another β-agonist, reverses the pulmonary pressor response to acute alveolar hypoxia[25,26]. Hexoprenaline was more effective than either normoxia or verapamil in reducing total pulmonary resistance in the chronically hypoxic calves. Speculatively, the greater effectiveness of hexoprenaline might have been due to enhanced intracellular sequestration and extrusion of activator calcium. It seems warrented to explore further the possibility that persistent elevation of activator calcium contributes to the sustained pulmonary vascular resistance in animals with chronic, hypoxia-induced pulmonary hypertension.

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Liver enzyme induction by the anion exchanger resin, Dowex 1 x 2, in the rat

F. E. Beyhl and E. Granzer[1]

Hoechst AG., Postfach 800320, D-6230 Frankfurt am Main 80 (Federal Republic of Germany), 14 January 1977

Summary. Peroral treatment of rats with the anion exchanger resin, Dowex 1 x 2, for 8 days leads to liver enlargement and increase of alkaline and acid phosphatases, glucose-6-phosphate dehydrogenase, malic enzyme, catalase, and the enzymes of microsomal drug metabolism in the liver. The sequestration of bile acids by this treatment mimics the biochemical effects of clofibrate- and phenobarbital-like drugs in the liver.

An interesting way for treating hypercholesterolemia is to administer to the patient a non-resorbable, macromolecular anion exchanger resin[2] which binds the intestinal bile acids during its passage through the gut and takes them out in the feces. Thus, more bile acids are excreted than normally[3-5] and the enterohepatic circulation of the bile acids is interrupted. Because the biosynthesis of bile acids from cholesterol is under negative feedback control by the bile acids reabsorbed from the gut[6,7], cholesterol catabolism is stimulated under anion exchanger resin treatment. The activity of cholesterol 7α-hydroxylase, a cytochrome-P-450-dependent mixed-function oxygenase in liver microsomes which determines the velocity of bile acid formation, is increased[8]. So more cholesterol is metabolized[9] and cholesterol blood level decreases[2,4,5]. Enhancement of bile acid biosynthesis is seen during bile drainage[8] when the enterohepatic circulation of bile acids is interrupted too. The inverse effect, i.e. decrease of bile acid formation, is seen when bile flow out of the liver is stopped either by bile duct ligation[10] or by α-naphthylisothiocyanate-induced cholestasis[11], so that the bile acids cannot leave the liver cell but inhibit their own biosynthesis.

Drug-metabolizing liver microsomal enzymes also depend on cytochrome P-450, and so bile duct ligation[10] and α-naphthylisothiocyanate treatment[11] diminish drug metabolism. Phenobarbital treatment stimulates both cholesterol catabolism[12] and drug metabolism[13]. So we did the following experiment in order to learn whether administration of the anion exchanger resin, Dowex 1x2®, to rats induces the biosynthesis of microsomal drug metabolizing liver enzymes.

Experimental. The experimental animals, male Wistar rats which had free access to food and drinking water, received Dowex 1x2 (chloride form, air-dried) for 8 days by stomach-tube in a daily dose of 500 mg/kg suspended in Tylose® solution; control rats received Tylose solution only. Each group consisted of 10 animals. 20 h after the

last application, all rats were killed by decapitation. The livers were excised rapidly and weighed; liver aliquots were frozen quickly in liquid nitrogen and stored at −20 °C until further handling. After thawing, the liver specimens were homogenized in ice-cold isotonic-KCl solution with a Potter-Elvehjem type homogenizer fitted with a teflon pestle. In the crude homogenates gained by this procedure, activities of alkaline phosphatase were measured by the method of Georgatsos[14], those of acid phosphatase by the method of Osanai and Rembold[15] (but with p-nitrophenylphosphate as the substrate), and of catalase by the method of Kaltenbach et al.[16] (catalase activities are expressed here as R values which are proportional to the enzyme activity: the inverse R value corresponds to the dilution of the homogenate at which half of the given H_2O_2 is decomposed by the enzyme content); of glutamate:pyruvate transaminase and glutamate dehydrogenase by Warburg's optical test[17,18], of NADH:cytochrome c oxidoreductase (mitochondrial cytochrome c reductase) by the method of Cleveland and Smuckler[19], and of succinate dehydrogenase by the method of King[20]. Protein contents were measured by the biuret method with bovine serum albumin as the protein standard. From aliquot of the crude homogenates, 12,000 × g-supernatants were prepared by centrifugation, in which the activities of α-glycerophosphate, malate, glucose 6-phosphate, 6-phosphogluconate, and isocitrate dehydrogenases and of malic enzyme were determined by Warburg's optical test[17,18], of guaiacol glycerol ether ring hydroxylase by the method of Beyhl and v. Kerekjarto (in preparation), and of aminopyrine N-demethylase, NADPH: cytochrome c oxidoreductase (microsomal cytochrome c reductase), and neotetrazolium reductase by methods described elsewhere in this journal[21]. Significance was calculated from the results by Student's t-test.

Results and discussion. The livers of the treated animals were enlarged, but protein contents remained constant (see table). The mitochondrial enzymes. glutamate and succinate dehydrogenases, and mitochondrial cytochrome c reductase, and most of the soluble enzymes, α-glycerophosphate, malate, isocitrate, and 6-phosphogluconate dehydrogenases and the transaminase, were unchanged. Therefore, we conclude that Dowex 1 x 2 treatment does not influence mitochondrial and cytosolic metabolisms, or at the most only parts of them.

Interestingly, some granule- and membrane-bound enzymes are increased (see table), e.g. alkaline and acid phosphatases. Also catalase activities are raised. This fact indicates that, during treatment with Dowex 1x2, peroxisomes (microbodies) proliferate as under treatment with hypolipidemic aryloxyalcanoic acids, e.g. clofibrate and 13 437-Su[22–25]. Dowex 1 x 2 treatment also resulted in an increase of drug metabolizing enzymes and microsomal reductases. Dowex 1 x 2 therefore induces the biosynthesis of the enzymes of the microsomal respiratory

Liver changes caused by Dowex 1 x 2 in the rat

	Control	Dowex	Significance
Relative liver weight (g of liver/100 g b.wt)	2.89±0.11 (100.0±3.8%)	3.31±0.19 (114.5±6.6%)	$p<0.0005$
Protein content (mg/g liver)	478±104	448±98	n.s.
Alkaline phosphatase (units/g liver)	0.413±0.104 (100.0±25.2%)	0.749±0.322 (181.4±78.0%)	n.s.
Acid phosphatase (units/g liver)	4.59±0.30 (100.0±6.5%)	5.30±0.23 (115.5±5.0%)	$p<0.001$
Catalase (R/g liver)	216±44* (100.0±20.4%)	872±192** (403.7±88.9%)	$p<0.0005$
Aminopyrin demethylase (units/g liver)	0.093±0.012 (100.0±13.0%)	0.192±0.057 (206.0±61.0%)	$p<0.0005$
Guaiacol glycerol ether ring hydroxylase (△ E/g liver)	21.8±12.8 (100.0±58.7%)	34.3±13.9 (157.3±63.8%)	n.s.
NADPH: cytochrome c oxidoreductase (units/g liver)	30.8±4.9 (100.0±16.0%)	43.8±8.9 (142.0±29.0%)	$p<0.005$
Neotetrazolium reductase (units/g liver)	30.2±5.5 (100.0±18.2%)	50.5±8.0 (167.2±26.5%)	$p<0.0005$
Glucose-6-phosphate deuydrogenase (uhits/g liver)	1.99±0.38 (100.0±19.1%)	2.96±0.51 (148.7±25.6%)	$p<0.001$
Malic enzyme (units/g liver)	0.290±0.017 (100.0±5.9%)	0.494±0.121 (170.3±41.7%)	$p<0.0005$

Means ± SD, N = 10. The numbers in brackets denote percentage of control. △ E: Extinction difference at 479 nm/min. *: N = 7; **: N = 8.

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chain, as do phenobarbital and a great number of other drugs[13, 21, 26]. The only difference is that these drugs are thought to enter the liver cell and to react with the microsomal systems[21, 26], whilst the anion exchanger resin does not because it is not absorbed by the intestine.
The activities of the soluble NADPH-generating enzymes, glucose 6-phosphate dehydrogenase and malic enzyme, are increased. This fits well with the greater demand for NADPH in order to feed the microsomal hydroxylation reactions. Malic enzyme is also increased with aryloxyalcanoic acids[24, 25]. Treatment of rats with 13 437-Su results in liver enlargement and increased activities of alkaline phosphatase, catalase, malic enzyme, aminopyrine demethylase, and microsomal cytochrome c and neotetrazolium reductases (Kief and Beyhl, unpublished results[24, 25]).

Dowex 1 x 2 treatment combines the effect of the aryloxyalcanoic acids (clofibrate-like drugs) and the microsomal inducers (phenobarbital-like drugs). It seems as if part of the effects of the aryloxyalcanoic acids can be ascribed to an interference with bile acid enterohepatic circulation (inhibition of enteral bile acid reabsorption, e.g.). On the other hand, induction of microsomal drug-metabolizing enzymes may be due to an overcome of bile acid negative feedback control of enzyme synthesis brought about by the inducing agent. So it is not necessary that a microsomal inducer should enter the liver cell; it can achieve its inducing action also by an indirect way, namely sequestration of bile acids in the intestinal tract.

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Improved treatment of organophosphate intoxication by use of scopolamine or dexetimide[1]

U. Bertram, A. Kasten, H. Lüllmann and A. Ziegler

Department of Pharmacology, University of Kiel, Hospitalstrasse 4–6, D-2300 Kiel (Federal Republic of Germany, BRD), 24 January 1977

Summary. Organophosphate intoxications have been treated by dexetimide plus obidoxime. Rabbits intoxicated by the 50–80fold LD_{50} of different organophosphates survived. Prophylactic application of dexetimide or scopolamine plus obidoxime increased the LD_{50} of DFP by a factor up to 180.

It is generally recommended to treat an intoxication with organophosphates – widely used as insecticides and in stock as chemical warfare agents – by repeated administrations of high doses of atropine. The treatment might be improved by the additional application of enzyme reactivators of the oxime type, e.g. pralidoxime and obidoxime. This treatment has to be supplemented by symptomatic procedures[2]. This commonly proposed therapeutic scheme often fails to be successful. The lack of success might be caused by the relative inability of atropine to counteract the intoxication of the central nervous system. We, therefore, directed our attention to cholinolytic drugs which more readily penetrate the blood-brain-barrier. The preliminary results obtained with scopolamine and dexetimide, a drug displaying strong central anticholinergic activity[3], will be reported. Dexetimide is the biologically active enantiomer of benzetimide (Tremblex®).
In a first series of experiments, the protective potency of dexetimide and of dexetimide plus reactivator was compared with that of atropine and scopolamine. Diisopropylphosphorofluoridate (DFP) was injected s.c. to mice (NMRI, 17–23 g b.wt) in a dose range of 24–4400 μmoles/kg b.wt. The LD_{50} amounted to 24 ± 0.14 μmoles/kg b.wt under control conditions. As shown in table 1, neither the cholinolytic drug nor obidoxime alone displayed a significant protection. The combined prophylactic treatment by atropine plus obidoxime resulted in a protection factor of 28 which is in accordance with other reports[4, 5]. An increase of the atropine dosage did not yield higher protection. Replacing atropine by the stronger centrally acting drugs scopolamine or dexetimide proved to result in far superior protection, as demonstrated by the protective factors of 183 and 180, respectively. In regard to scopola-

Table 1. Toxicity (LD_{50}) of DFP in mice after pretreatment by cholinolytics and obidoxime

μmoles/kg b.wt	Obidoxime (mol.wt 359) μmoles/kg b.wt	LD_{50} DFP (mol.wt 184) μmoles/kg b.wt	Protection factor*
Atropine (mol.wt 347)			
20	–	45	1.9
10	10	280	12
10	100	680	28
Scopolamine (mol.wt 384)			
20	–	42	1.8
10	10	610	25
10	100	4400	183
Dexetimide (mol.wt 399)			
20	–	38	1.68
10	10	960	40
10	100	4360	180
–	10	44	1.7
	100	62	2.3

The LD_{50} in controls was 24 μmoles ± 0.14/kg b.wt. The drugs were applied s.c., the protecting compounds were injected 15 min before DFP administration.

*Protection factor = $\frac{LD_{50}\ \text{pretreatment}}{LD_{50}\ \text{controls}}$.

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