In vitro protein synthesizing activity of monkey lung subcellular fractions 4 months after intratracheal administration of silica

Subcellular particulate fraction	Soluble fraction	Incorporation of (^{14}C)-amino acids into protein	
		cpm/mg particle RNA	Control (%)[a]
Ribosomes	Cell sap		
	Silica treated	2880	45
	Control	3103	49
	pH 5 enzyme		
	Silica treated	22,055	66
	Control	24,561	73
Rough membranes	Cell sap		
	Silica treated	1770	48
	Control	1992	54
	pH 5 enzyme		
	Silica treated	9279	65
	Control	8180	57

[a] Control is the incorporation obtained with control particulate and soluble fractions.

The reduced overall protein synthesis, at a time when fibrogenesis is actively taking place, implies effects which are not discernible by histological examination. It remains to be established what effect the diminished protein synthesis has on the functional integrity of the lung[8].

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Zusammenfassung. Die Proteinsynthese der Lungenribosomen (frei oder membrangebunden) vom Affen nach Injektion von Silika wurde untersucht. Die Abnahme der Proteinsynthese nach 4 oder 6 Monaten ist nicht von löslichen Faktoren abhängig.

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Lathyrogenic Activity of 2 (2-Cyanoethyl)-3-isoxazolin-5-one from *Lathyrus odoratus* Seedlings

Several isoxazolin-5-one derivatives have recently been isolated from *Lathyrus odoratus* (sweet pea) seedlings[1,2]. One of these, 2 (2-cyanoethyl)-3-isoxazolin-5-one, component VIII, can be regarded as a new derivative of β-aminopropionitrile (BAPN), the well-known active principle[3–5] of the substance γ-GluBAPN in sweet pea seeds that produces changes in collagen in a variety of species including chicks, turkeys, baboons, rats and tadpoles. In the rat, effects of BAPN may include skeletal deformities, hernia and aortic rupture. In this report the isoxazolin-5-one derivative VIII has been examined in the weanling rat for signs of lathyrogenic activity.

Materials and methods. Component VIII was isolated from 10-day-old *L. odoratus* seedlings by extraction with ethanol and ion exchange chromatography as described[2]. Weanling white male Sherman rats, 33–38 g body wt., were used. The basal diet was Teklad 4% Mouse/Rat diet (Animal Feeds, Inc., Bronx, N.Y .) containing 24% crude protein, 4% fat, 6% crude fibre, supplemented with vitamin A, palmitate, irradiated dried yeast, niacin, calcium pantothenate, riboflavin, menadione, vitamin B_{12}, 1% calcium carbonate, 0.5% dicalcium phosphate, 1% salt, and traces of manganese oxide, copper oxide, cobalt carbonate, iron carbonate, zinc oxide, and calcium iodate. Comp VIII was incorporated as 0.4% of the basal diet. A control group received the equivalent (0.3%) of BAPN·HCl (Calbiochem. Calif.) and another control group received the unsupplemented diet. All diets were consumed ad libitum. After 18 days, rats were x-rayed under ether anesthesia.

After 10 days, urine was collected in metabolic cages over a 24-h period in the presence of thymol. Excreted BAPN was detected by electrophoresis of untreated urine on Whatman No. 3 paper in 0.1 *M* pyridine acetate buffer, pH 5.6, for 3 h at 10 V/cm. The sample contained material that travelled with the mobility of the authentic basic BAPN and gave its characteristic green color when treated with ninhydrin[6]. Excreted BAPN was determined quantitatively by chromatography on a Beckman Spinco analyzer[7] on the 15-cm column with Type 15A resin and 0.35 *N* sodium citrate buffer, pH 6.5, at 30 °C. The

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Skeletal deformities produced by 2(2-cyanoethyl)-3-isoxazolin-5-one from *L. odoratus*. Rat on left, normal; rat on right, experimental.

ninhydrin color constant of BAPN was 2.84; the elution volume, 173 ml. The stability of VIII in urine for 24 h was examined by quantitative analysis for BAPN. Excreted thiocyanate was determined colorimetrically as its red complex with ferric ion[8].

Results and discussion. Dietary administration of VIII to the weanling rat resulted in growth retardation and skeletal abnormalities. X-ray, which provides reliable criteria on lathyrism[9], showed mild scoliosis and periosteal new bone in humerus, femur, and pelvis with flaring of the ischeal and pubic rami of the pelvis and increased shaft diameter of the femur. In the BAPN-fed rats rib-cage deformity was somewhat more pronounced, scoliosis more generalized, and shortening and widening of long bones somewhat increased.

The rats fed BAPN excreted 23 μmoles/24 h of BAPN, which is in general accord with Lalich's observation that a significant part (40%) of administered radiolabelled BAPN is excreted unchanged in 20 h[10]. The rat consuming the equivalent of VIII excreted 14 μmoles/24 h free BAPN. VIII is labile to intense UV-irradiation or strong alkali (0.01 M NaOH) when it liberates BAPN[11], and it now appears also to be metabolically labile in the rat. If excretion of BAPN is roughly indicative of dosage, the quantity of BAPN excreted following ingestion of VIII might correspond to a 0.12% BAPN diet. This level is likely to be lathyrogenic on the basis of the observations of femur fibrosis and vertical column deformity made by BACHHUBER et al.[3] with a 0.15% BAPN diet and of slight effects at 0.1%[4]. The activity of VIII can therefore probably be attributed to the formation of BAPN. A free primary amino or similar basic grouping is generally considered a structural requirement for osteolathyrogenic activity[3,4,12]. The two other active bound forms of lathyrogens, γ-GluBAPN[9,13] and N-methyleneamino-acetonitrile[12], might also be expected to be sufficiently labile biologically to liberate the lathyrogen bearing a free amino group.

Thiocyanate excretion, which is elevated in the BAPN-fed rat[14], is likewise elevated in the rat fed VIII: 8.2 μmoles as compared to 1.6 μmoles/24 h for untreated controls.

L. odoratus seedlings have been observed to have 0.7% isoxazolin-5-one (VIII) and 1.8% γ-GluBAPN[15]. The present demonstration of lathyrogenic activity with a diet of 0.4% VIII strengthens the suggestion that the seedlings of this species, in addition to the seeds, have the potential of being highly lathyrogenic.

Résumé. L'administration au rat de 2(2-cyanoethyl)-3-isoxazolin-5-one (comp. VIII), un composant des jets de *Lathyrus odoratus*, cause un retardement de la croissance ainsi que des anomalies du squelette, caractéristiques de la substance lathyrogène β-aminopropionitrile (BAPN). L'excrétion du BAPN suggère que l'activité lathyrogène de VIII est dûe à la libération du BAPN in situ.

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