Comparative Effects of an Osteolathyrogen and a Neurolathyrogen on Brain and Connective Tissues[1] (34818)

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In a search for a biochemical basis of experimental neurolathyrism, it was of interest to see whether changes in activities of acid hydrolases, of presumed lysosomal origin, could be detected in brain tissue of rats challenged with β,β'iminodipropionitrile (IDPN). It had been shown previously in this Laboratory (1) that administration of the osteolathyrogen β-aminopropionitrile (BAPN) resulted in significant increases in the acid hydrolase activity of granulomatous tissue induced by subcutaneous implantation of polyvinyl plastic sponge, and it was thus, of further interest to compare the effects of the osteolathyrogen and the neurolathyrogen on both brain and connective tissues.

Young rats treated with IDPN exhibit severe neuromotor dysfunction, clinically characterized by Selye (2) as the "ECC syndrome" (excitement with choreiform and circling movements). Histologic examination of the central nervous system of such animals reveals necrosis of Purkinje cells in the cerebellum and shrinkage of Betzschen cells of the motor cortex (3). The cell bodies of the motor neurons located in the anterior and ventrolateral horn of the spinal cord are found to be two to three times larger than normal cells and have been referred to as ghost cells (4). Few biochemical studies have been made on the effects of IDPN administration on the central nervous system. Vivanco *et al.* (5) reported increases in γ-aminobutyric, aspartic, and glutamic acids, but other investigators have not found such increases (6, 7). There have been occasional reports (3, 8) of minor skeletal lesions resulting from prolonged administration of IDPN; however, Levene (9) and others did not consider this chemical to be an osteolathyrogen, but only a neurolathyrogen.

BAPN, an analog of IDPN, does not produce the ECC syndrome. Its toxicity is apparently limited to connective tissues (10). The administration of BAPN results in the accumulation of soluble tropocollagen molecules which are unable to form stable intra- and intermolecular crosslinks because of a deficiency in aldehyde groups (11). The extent of this increase in soluble collagen is used as a measure of the degree of osteolathyrism. The relationship between the pathology of the ECC syndrome and of osteolathyrism is not known, and this study was undertaken to determine whether any such relationship exists.

Methods. Subcutaneous granulomas were induced by implanting a polyvinyl plastic sponge (weighing 110 $\pm$ 5 mg) subdermally on the dorsum of male rats of the Holtzman strain (initial weight 65–75 g). In each experiment, the animals were separated into three groups of from 8–10 animals each. The control group received daily subcutaneous injections of 1 ml of sterile saline. The second group received daily subcutaneous injections of 45 mg of IDPN in 1 ml of saline, and the third groups was injected intraperitoneally with 2 ml of saline containing 100 mg of BAPN for each 100 g of body weight. These doses of IDPN and BAPN had previously been established as being optimal for producing the ECC syndrome and osteolathyrism, respectively. The animals were pair-fed so that similar weight changes would be obtained in each group.

After 15 days in one experiment and after 20 days in another, the rats were decapitated and the whole brain removed, weighed, and

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TABLE I. Collagen Content of Sponges.

Treatment	Collagen (mg/sponge)			
	NaCl	Citrate	Total soluble	Insoluble
Saline	0.89 ± 0.20[a]	0.57 ± 0.19	1.46	8.99 ± 1.11
IDPN	1.09 ± 0.25	0.85 ± 0.25	1.94	5.42 ± 1.44
BAPN	2.21 ± 0.65	0.34 ± 0.57	2.55	5.82 ± 1.59

[a] Standard deviation.

TABLE II. Collagen Content of Sponges.

Treatment	Collagen (mg/sponge)			
	NaCl	Citrate	Total soluble	Insoluble
Saline	1.18 ± 0.29	1.16 ± 0.13	2.34	14.09 ± 2.67
IDPN	2.05 ± 0.19	1.47 ± 0.24	3.52	8.02 ± 1.55
BAPN	2.30 ± 0.21	2.49 ± 0.14	4.79	7.37 ± 2.15

transferred to ice-cold 0.25 *M* sucrose (1:10 w/v). The tissue was dispersed with a homogenizer of the Potter-Elvehjem type equipped with a Teflon pestle. The homogenized samples were centrifuged for 10 min at 600*g*, and the supernatant fluid was recovered for the enzyme determinations.

All enzyme assays were carried out at 37°, and in the presence of Triton X-100 to rupture any intact lysosomal membranes. Cathepsin activity was determined at pH 3.6 by a slight modification of the Anson hemoglobin procedure (12). Solubilized protein was equated to tyrosine absorbance at 280 mμ. β-Glucosaminidase was determined with 1.1×10^{-3} *M* *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide as substrate (0.2 *M* citrate–phosphate buffer, pH 4.4), and acid phosphatase with 1.4×10^{-3} *M* *p*-nitrophenyl phosphate (0.2 *M* acetate buffer, pH 4.5). Liberated *p*-nitrophenol was measured at 400 mμ at pH 12. Excised sponges were sequentially extracted with 1 *M* NaCl and 0.5 *M* citrate (pH 3.6), and the extracts and insoluble residue analyzed for collagen hydroxyproline as described by Nimni and Bavetta (13).

Results. Table I summarizes the changes in sponge granuloma collagen resulting from the administration of IDPN and BAPN for 15 days. Table II gives the data obtained after the administration of these lathyrogens for 20 days. The total amount of soluble collagen was significantly increased in the treated animals, while the amount of insoluble collagen was significantly decreased. BAPN was somewhat more effective than IDPN in causing these changes. Both chemicals, however, produced changes of a magnitude to qualify them as potent "osteolathyrogens" (10).

Table III presents a summary of the data obtained from the brain enzyme assays of the 15-day experiment. In all cases, the treated animals showed less enzyme activity than the controls, although the differences were not statistically significant. However, such decreases have been consistently found in other experiments.

All of the animals treated with IDPN exhibited a pronounced ECC syndrome, whereas none of the BAPN-treated animals exhibited any such symptoms.

TABLE III. Enzyme Activity of Brain Extracts.

Enzyme	Control	IDPN	BAPN
Acid phosphatase[a]	87 ± 13	77 ± 8	62 ± 15
Beta-glucosaminidase[a]	57 ± 13	56 ± 5	42 ± 5
Cathepsin[b]	39 ± 7	34 ± 5	31 ± 12

[a] Activity expressed as μmoles *p*-nitrophenol liberated/hr/g brain.

[b] Activity expressed as μmoles tyrosine liberated /hr/g liver.

Discussion. Accumulation of soluble collagen, in both hard and soft connective tissues, is often used as the criterion of experimental osteolathyrism. From these results, it is apparent that administration of the "neurolathyrogen", IDPN, results in excessive accumulation of soluble collagen in sponge-induced granulomas, and on this basis it can also be classified as an osteolathyrogen. The effect with IDPN was not so pronounced as with BAPN, but on the basis of molecular weight, the BAPN dose was almost twice that of the IDPN. Whether IDPN has a similar effect on other soft connective tissues has not been determined, although Levene (9) reported no increase in soluble collagen of IDPN-treated chick embryos, using relative viscosity of saline extracts as the criterion of the amount of collagen solubilized. The effect of IDPN on hard tissues is apparently slight, as skeletal deformities have been reported to be minor (3, 8). Whether IDPN and BAPN have similar modes of action on mesenchymal tissue, such as the sponge-induced granuloma, remains to be determined. However, the finding that IDPN can induce an experimental condition affecting both connective and nervous tissues offers a new approach to an understanding of the molecular aspects of lathyrism.

The mode of action of IDPN as a neurolathyrogen is not understood at present. It does not affect the entire brain and spinal cord but is apparently specific for motor areas. The present studies failed to help clarify the situation, as the brain acid hydrolases showed no statistically significant differences in the experimental and control animals. The small but reproducible decreases in activity that were found are probably not causally related to the neurolathyritic symptoms, since the decreases were also found in the BAPN-treated animals, which did not exhibit the ECC syndrome.

Summary. β,β'-Iminodipropionitrile (IDPN), previously thought to be exclusively a neurolathyrogen, was shown to be almost as potent an osteolathyrogen as β-aminopropionitrile (BAPN), as judged by the accumulation of soluble collagen in sponge-induced granulomas of IDPN-challenged rats. No significant changes in cathepsin, β-glucosaminidase, and acid phosphatase activities could be demonstrated in brain extracts of rats challenged with either IDPN or BAPN.

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