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DIPHOSPHOPYRIDINE NUCLEOTIDE SYNTHESIS IN BRAIN FOLLOWING INJECTION OF VARIOUS COMPOUNDS

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Abstract—Diphosphopyridine nucleotide synthesis in brain has been investigated following intraperitoneal and/or intrathecal injections of nicotinamide, nicotinic acid, reserpine and azaserine.

The findings described in this note give conclusive evidence that pyridine nucleotide synthesis is an active process in brain as well as in liver, although it is much easier to alter DPN turnover in liver than in brain.

A LARGE rise in the DPN concentration of mouse liver following intraperitoneal injection of nicotinamide was described some 8 years ago by Kaplan *et al.*[1] By contrast, the pyridine nucleotide level of the brain was found to undergo only a minor change when the compound was administered by the same route. Wilson *et al.*[2] have observed a decrease of tissue DPN levels by iproniazid but the reduction caused by this compound in the DPN level in brain was much less marked than that found in liver.

When reserpine is injected intraperitoneally to mice before nicotinamide, the DPN elevation in the liver is extended to periods beyond 24 hr, while the rise after a single dose of nicotinamide is followed by a rapid decrease in the DPN level to the control concentration.[3] DPN synthesis in liver may be also greatly altered by injecting azaserine: extremely low levels of the liver DPN are attained in a short period of time.[4, 5]

In the present investigation, nicotinamide, nicotinic acid, reserpine and azaserine* have been used to assess whether changes obtained in the DPN turnover in the liver could be duplicated in brain. With particular reference to reserpine, it may be readily seen that chemical studies on brain are more relevant to the pharmacological action of the compound than findings on liver chemistry.

Experiments were carried out using groups of three adult male albino mice, each mouse weighing from 18 to 23 g. Feed was withdrawn at the time of injection. Nicotinamide (500 mg/kg), nicotinic acid (50 mg/kg) and azaserine (200 mg/kg) were injected intraperitoneally or intrathecally as neutralized solutions in a volume of 0·04 ml or 0·02 ml. Reserpine (10 mg/kg) was given by intraperitoneal injection in the solvent mixture used by Burton *et al.*[3] DPN was extracted from the tissues by homogenization with 5 vols of cold 5% trichloroacetic acid and measured spectrophotometrically with yeast alcohol dehydrogenase and ethanol.[4] Acid-soluble compounds were determined by extinction measurements at 260 mμ.

* Nicotinamide and nicotinic acid were purchased from California Corporation for Biochemical Research (Los Angeles, Calif., U.S.A.). Reserpine was a gift of Ciba Pharmaceutical Products Inc. (Summit, New Jersey, U.S.A.) and azaserine a gift of Parke, Davis & Co. (Detroit, Michigan, U.S.A.).

Intrathecal injections of nicotinamide are responsible for the same curve of DPN elevation in liver as when the precursor is given intraperitoneally. As already described by Kaplan *et al.*,[1] liver and brain exhibit the peak value of DPN level at from 8 to 12 hr after injection, when nicotinamide is given intraperitoneally. In contrast with this, following intrathecal injections, brain DPN attains its peak value much earlier, i.e. within 4 hr, and returns very quickly to the control concentration or even below (curve A of Fig. 1). This faster increase in the DPN level in the brain is not found when

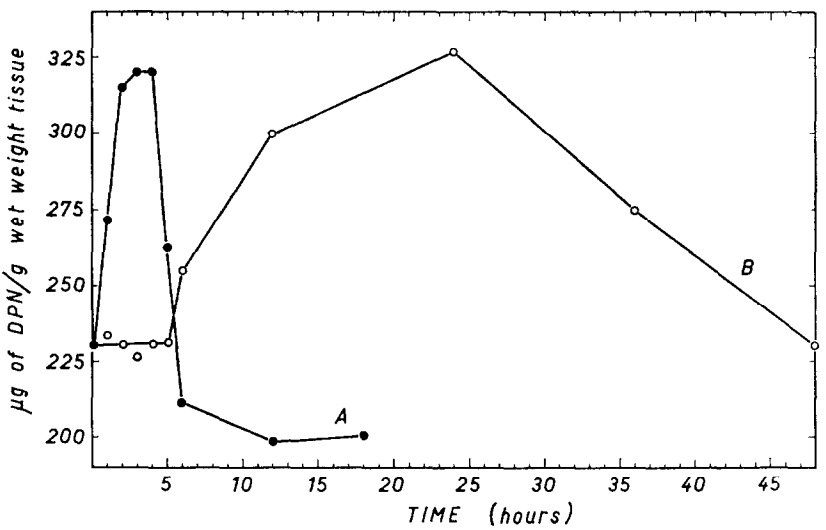

FIG. 1. Effect of reserpine (10 mg/kg body weight) upon the level of diphosphopyridine nucleotide in mouse brain. Reserpine was injected intraperitoneally and nicotinamide intrathecally. Curve A refers to data obtained from animals that had received only nicotinamide; curve B to data obtained from animals that had received intraperitoneal reserpine 4 hr before intrathecal nicotinamide. For other details, see the text.

nicotinic acid is given as a precursor intrathecally. In this instance, DPN concentration in brain attains its peak value at the sixth hour after the injection, while liver DPN climbs to the maximum within 2 hr. Moreover, the elevation in liver is much more consistent than in brain (150 per cent against 15 per cent). This again points to a significant difference in the metabolic stability of DPN in brain and liver.

Curve B in Fig. 1 shows how the peak value of the increase in brain DPN is greatly delayed, though it attains the same level, when reserpine is administered by intraperitoneal injection 4 hr before the intrathecal injection of nicotinamide. Curve B for brain DPN duplicates the findings of Burton *et al.*[3] in liver after the intraperitoneal injection of reserpine and nicotinamide. By contrast the intraperitoneal injection of the two compounds does not result in any consistent change of DPN levels in brain.

As mentioned before, the intraperitoneal injection of azaserine is responsible for a sharp decrease of liver DPN.[4, 5] This could not be duplicated in brain where DPN level did not undergo any change after intraperitoneal administration. Intrathecal injections of azaserine, however, caused a consistent decrease of DPN (from 230 to

130 μg/g wet weight) in brain, and also a decrease was observed, after intrathecal injection, in liver (Fig. 2). Both brain and liver exhibited a net decrease in the content of acid-soluble compounds (−14 and −18 per cent, respectively, at the third hour after injection). Conversely, the intraperitoneal injection caused a decrease of E_{260} of liver but not of brain extracts.

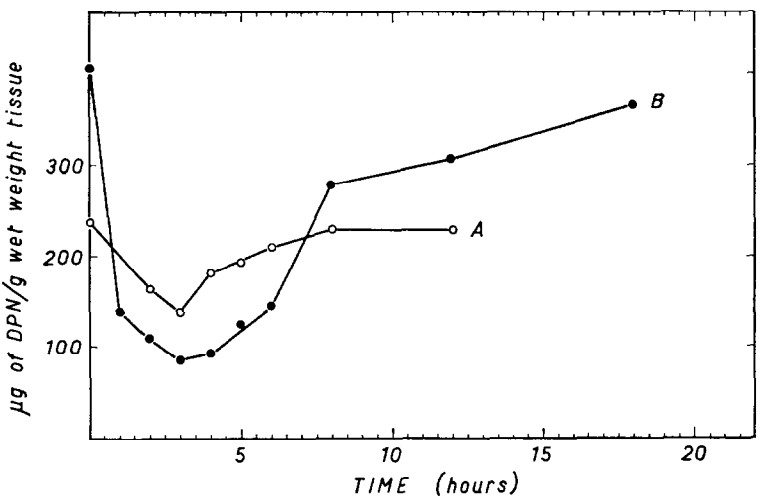

FIG. 2. Effect of azaserine (200 mg/kg body weight) upon the level of diphosphopyridine nucleotide in mouse brain (curve A) and liver (curve B). The compound was injected intrathecally.

The findings described in this note give conclusive evidence that pyridine nucleotide synthesis is an active process in brain as well as in liver, although it seems much easier to alter DPN turnover in liver than in brain.

The diverse time curve of DPN elevation after intrathecal or intraperitoneal injection of nicotinamide suggests that an investigation of the rate of penetration of labelled nicotinamide through the blood–brain barrier and its metabolic pathways in brain would be useful. These experiments are in progress. That reserpine is responsible for a delay in the elevation of brain DPN only when nicotinamide is injected intrathecally cannot be easily explained at present. This finding points, however, to a possible effect of reserpine on turnover of brain DPN.

The retarded and slight elevation of brain DPN after intrathecal injections of nicotinic acid contrasts with the earlier and greater elevation in liver, and may be regarded as an indication that pyridine nucleotide synthesis in brain does not follow the same pathway as in liver.

Finally, it is worth mentioning that the reduction in the level of brain DPN and acid-soluble u.v. absorbing compounds after intrathecal injection of azaserine is paralleled by deep electrographic changes as compared to control mice receiving intrathecal injection of the vehicle.[7]

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REFERENCES

1. N. O. KAPLAN, A. GOLDIN, S. R. HUMPHREYS and F. E. STOLZENBACH, *J. biol. Chem.* **226**, 365 (1956).
2. D. WILSON, M. KUEHNER and G. WOLFF. Unpublished data; see R. M. BURTON, *Inhibition in the Nervous System and Gamma-Aminobutyric Acid* (Edited by E. ROBERTS *et al.*), p. 249. Pergamon Press (1960).
3. R. M. BURTON, N. O. KAPLAN, A. GOLDIN, M. LEITEMBERG, S. R. HUMPHREYS and M. A. SODD, *Science* **127**, 30 (1958).
4. S. A. NARROD, T. A. LANGAN JR., N. O. KAPLAN and A. GOLDIN, *Nature, Lond.* **183**, 1674 (1959).
5. S. A. NARROD, V. BONAVITA, E. R. EHRENFELD and N. O. KAPLAN, *J. biol. Chem.* **236**, 931 (1961).
6. M. M. CIOTTI and N. O. KAPLAN, *Methods in Enzymology* (Edited by S. P. COLOWICK and N. O. KAPLAN) Vol. III, p. 891. Academic Press, New York (1957).
7. V. BONAVITA, N. BONASERA and M. ZITO. Unpublished results.