Reserpine (Serpasil CIBA) was injected intraperitoneally whilst control animals received an equivalent volume of saline.

TKT activity was determined in whole liver homogenate by the method described by Kenney.[7] In order to investigate better the enzyme-coenzyme saturation, the enzymic activity was measured with or without added pyridoxal-5-phosphate (Py).

RESULTS AND CONCLUSION

In Table 1 are presented the results of a dose-effect study. As it can be seen, reserpine increases the level of TKT several times: maximal elevation is obtained, in the time considered in this study, with 5 mg/kg of the alkaloid while a clear-cut response is observed with 1 mg/kg. Further results, not reported here, indicate that, with the smallest dose of reserpine, induction reaches its maximum after a longer delay.

The rise of TKT activity induced by reserpine is a long lasting effect as shown by the results presented in Fig. 1. With the rather high dose of reserpine used in this experiment, very high levels of TKT are still observed after 72 hr, and the enzyme activity is back to normal values after four days only.

This time curve is quite different from that which may be observed after a single injection of hydrocortisone[8] and differs as well from the time response of tryptophan pyrrolase to reserpine.

The fact that TKT activity is still elevated when both the pharmacological and the neurohormonal effects of reserpine have disappeared, is not easily understandable and may depend on the biochemical characteristics of the enzyme.

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The separation of synaptic vesicles from disrupted nerve-ending particles*

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WHEN guinea-pig brain is homogenized in 0·32 M sucrose, the nerve endings are torn away from their pre- and postsynaptic attachments to form nerve ending particles (NEPs), which can be isolated as a relatively pure fraction by differential and density gradient centrifugation.[1-3] This fraction is rich in bound acetylcholine (ACh),[4,5] choline acetylase (ChA)[4], hydroxytryptamine (HT)[5,6] and other amines. It also accounts for the 20 per cent of brain lactic dehydrogenase (LDH) which remains particle bound[7,8] on homogenization.

Using LDH as a marker for the soluble cytoplasm entrapped with NEPs, Johnson and Whittaker[7,8] compared the release of LDH and ACh brought about by disruptive procedures. Suspension of NEPs in hypo-osmotic solutions liberated up to 80 per cent of LDH but only 50 per cent of ACh,

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pointing to the partial survival, in these preparations, of a second permeability barrier to the outward diffusion of ACh not operative in the case of LDH. It was felt that this barrier could be the walls of the synaptic vesicles (SVs), the characteristic cytoplasmic organelles of the nerve ending proposed[9, 10] as the actual storage sites of ACh in cholinergic nerves. This view was supported by electron microscopic examination of negatively stained[11] hypo-osmotic suspensions, which showed the presence of many disrupted NEPs, clumps of SVs and some isolated SVs[8]. Free, mono-dispersed SVs have now been isolated from these preparations by submitting them to density gradient separation. Usually a P_2 fraction[4, 5] was used: this was prepared at a lower speed (10,000 $\times$ g for 20 min) than previously to reduce microsomal contamination. It contained besides NEPs, free mitochondria, myelin fragments and some microsomes. Similar results were obtained with a B fraction,[3] containing mainly NEPs. The preparations were uneserinized so that any free ACh released during treatment was destroyed by the cholinesterase present. After suspending the P_2 pellet in water (2 ml/g original tissue), myelin fragments and the larger mitochondria together with some intact and partially disrupted NEPs were removed by centrifuging at 10,000 $\times$ g for 20 min; the supernatant was transferred to a density gradient consisting of equal volumes of 0·4, 0·6, 0·8, 1·0 and 1·2 M sucrose, and the whole centrifuged at 53,500 $\times$ g for 2 hr. After spinning, the density gradient was sliced into seven fractions. The top fraction (O) consisted of an optically clear water layer which had originally contained the disrupted NEPs. The second fraction (D) was the now slightly hazy layer of 0·4 M sucrose immediately below O. The third (E), fourth (F), fifth (G) and sixth (H) fractions corresponded to particulate layers lying between sucrose layers of increasing molarity. The last fraction (I) was a pellet. The results of electron microscopic examination and assay are shown in Table 1.

TABLE 1. DISTRIBUTION OF LDH, ChA, ACh AND TOTAL NITROGEN IN FRACTIONS PREPARED BY DENSITY GRADIENT SEPARATION OF WATER-TREATED P_2 FRACTION

Fraction	Density (M sucrose)	LDH[7]	ChA[13]	ACh[14]	Total N	Morphology[11]
		(% recovered activity)				
O	0	80	67	2	32	Particle-free
D	0·4	9	9	30	10	SVs, occasional microsomes
E	0·4–0·6	7	1	11	7	Microsomes, some free and clumped SVs
F	0·6–0·8	1	8	10	11	NEP ghosts, myelin fragments
G	0·8–1·0	1	5	14	9	NEP ghosts, membrane fragments
H	1·0–1·2	1	6	26	15	Damaged NEPs, NEP ghosts
I	1·2	1	4	7	16	Small mitochondria, some shrunken NEPs
Recovery(%)		72	88	85	86	

NEP ghosts are the outer envelopes of NEPs containing few or no SVs. The membrane fragments in G were non-vesicular and resembled postsynaptic thickenings.

Composition of water supernatant from P_2/vol equivalent to 1g of tissue (units of enzyme activities in brackets): LDH (ΔE_{340}/min), 21·4; ChA (μmole ACh synthesized/hr), 0·4; ACh, 0·75 mμmole; total N, 2·3 mg (by micro-Kjeldahl). References are to methods. Specific ACh activities (mμmole/mg N): water supernatant, 0·33; fraction D, 1·09; fraction H, 0·57.

The most important finding was that fraction D, which had the highest ACh activity and specific activity (compare cols. 5 and 6), consisted almost entirely of mono-dispersed particles about 500 Å in diameter, identical in appearance with synaptic vesicles. ACh was bimodally distributed; a second peak occurred in H which consisted mainly of partially disrupted NEPs. By contrast, LDH, representing soluble cytoplasm released from disrupted NEPs, was recovered mainly in fraction O. A dummy run with bovine serum albumin showed that 76 per cent of this soluble protein also remained in the top fraction during centrifuging. ChA had a distribution resembling LDH rather than ACh, suggesting that ACh is synthesised in the nerve-ending cytoplasm, rather than the SVs.

The distribution of HT differed from that of ACh in that HT was found in all fractions with a peak in *H*. Dummy runs with free HT showed that this small molecule diffused throughout the density gradient in 2 hr. HT is known to be less firmly bound that ACh[6], and the presence of HT throughout the gradient probably represents diffusion of free HT from a binding site in *H*.

The water supernatant from which the fractions were prepared corresponds to the combined M_2 and M_3 fractions of De Robertis *et al.*[12] In electron micrographs of this fraction areas were sometimes found containing free SVs relatively uncontaminated by disrupted NEPs and other components, but all the particles listed in Table 1 could be readily identified in adjacent areas.

A full account of this work is in course of preparation.

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