—SHORT COMMUNICATIONS

SUBSTANCE P IN THE CENTRAL NERVOUS SYSTEM
OF RABBITS : UPTAKE SYSTEM DIFFERS
FROM PUTATIVE TRANSMITTERS

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Neurophysiological and neurochemical data have suggested that substance P may function as a neurotransmitter at synapses in the central nervous system, particularly in primary sensory neurons (1, 2). Mechanisms responsible for terminating the action of substance P at the synapse are however, unknown. Benuck and Marks (3) have partially purified the enzyme responsible for inactivation of substance P in rat brain. However, extensive research with the biogenic amines has suggested that enzymatic inactivation of a neurotransmitter is an exception rather than the rule (4). Several putative transmitters are taken up into nerve terminals through a specific high affinity uptake system which appears to account for their inactivation process. Although presence of a high affinity uptake system is not a criterion necessary for transmitter identification, such would greatly favor the candidacy of a compound for the transmitter (4).

A bovine hypothalamus substance P, Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met, was synthesized by the conventional method described in a previous paper (5). The synthetic substance P was labelled with tritium by a catalysed gas-exposure method, using platinum as the catalyst (6, 7). To remove the catalyst, unstable generally labelled compounds, labile tritium and tritiated impurities which arise during irradiation, the irradiated substance P was lyophilized eight times. The purity of the labelled compound was checked as follows: 1) When the compound was dansylated by the method of Gray and Hartley (8) and was submitted to thin layer chromatography of silica gel, a single spot was observed. Furthermore, the spot coincided with the peak of radiochemical activity determined by a scan of thin layer chromatogram. 2) The biological activity on guinea pig ileum was approximately 80% of that of the parent compound. 3) A mixture of an aliquot of labelled and parent substance P was submitted to gel chromatography using a Sephadex G-15 column. When the column was screened for both guinea-pig ileum contracting activity and radioactivity the patterns of both were similar. These results indicated that the labelled substance P was of high purity. The compound is a powder, with a specific activity of 1.31 Ci/mmole

and a molecular weight of 1340.

Rabbits weighing 1.9–2.6 kg of both sexes were sacrificed and mesencephalon, diencephalon and spinal cord were minced and homogenized in 9 vol. of ice cold 0.32 M sucrose using a Teflon pestle. The crude mitochondrial P₂ fraction was prepared by the method of Segawa and Kuruma (9). Electron microscopic examination revealed that the fraction was composed of intact synaptosomes, myelin and mitochondria (10). The fraction was suspended in modified Krebs-Ringer solution bubbled with 95% O₂-5% CO₂ for 10 min. This solution was composed of 118 mM NaCl, 4.7 mM KCl, 1.9 mM CaCl₂, 0.5 mM MgCl₂, 25.0 mM NaHCO₃ and 11.1 mM glucose. The suspension (0.7–1.5 mg protein/ml) was divided into 0.9 ml units, each of which was transferred into 10 ml polyethylene tubes. After pre-incubation for 10 min at 37°C, 0.1 ml of ³H-substance P (final concentration 2.3 × 10⁻⁸–2.3 × 10⁻⁶ M) was added and a further incubation was carried out at 37°C for intervals varying from 1 to 20 min. At the end of the incubation period, 6 ml of the cold saline were added to the reaction mixture and centrifugation at 11,500 × g was carried out for 20 min at 4°C after which the supernatant fluid was decanted. The pellet of the fraction was resuspended in 6 ml of the cold saline and was re-centrifuged at 11,500 × g for 20 min. The washing was repeated. The residual radioactivity in the final washing was found to be essentially negligible. A 0.1 ml portion of 1 N NaOH solution was added to the pellet, incubation was carried out at 37°C for 30–60 min and the preparation was then left overnight. After neutralization with 0.1 ml of 1N HCl solution, radioactivity was determined in 10 ml of Bray's solution by a Packard Tri-Carb liquid scintillation counter. Counting efficiency was 20–25%. The protein was estimated by the Folin reagent method of Lowry et al. (11).

The results are presented in Table 1. When crude mitochondrial P₂ fraction was incubated with ³H-substance P for 1 or 10 min, substance P was to some extent taken up into the fraction. However, since the fraction took up almost the same amount of substance P

TABLE 1. Uptake of ³H-substance P into crude mitochondrial P₂ fraction from rabbit brain and spinal cord

Concentration of ³ H-substance P (M)	Incubation time (min)	Mesencephalon cpm/mg protein (37°C–4°C)	P/M*	Diencephalon cpm/mg protein (37°C–4°C)	P/M*	Spinal cord cpm/mg protein (37°C–4°C)	P/M*
2.3 × 10 ⁻⁶	1	1499 (3)	0.07	—	—	4317 (3)	0.20
	10	5231 (3)	0.24	—	—	0 (3)	0.00
2.3 × 10 ⁻⁷	1	569 (3)	0.27	—	—	—	—
	10	963 (3)	0.45	—	—	—	—
2.3 × 10 ⁻⁸	1	51 (3)	0.24	88 (1)**	0.41	131 (1)	0.61
	10	212 (3)	0.99	217 (1)	1.02	0 (1)	0.00
2.3 × 10 ⁻⁶ (3 mM ATP)	1	5394 (3)	0.25	—	—	0 (1)	0.00
	10	3390 (3)	0.16	—	—	0 (1)	0.00

* Pellet/medium ratio = $\frac{\text{cpm/100 mg pellet protein}}{\text{cpm/ml medium}}$

** Five min incubation.

Figures in parentheses indicate number of observations.

both at 4°C and 37°C, the net uptake at 37°C, calculated by subtracting the value at 4°C from the value at 37°C was very small or nil. Thus, the pellet/medium ratio, calculated from the net uptake at 37°C scarcely exceeded 1. The uptake was not enhanced either by prolonging incubation time to 20 min or by adding 3 mM ATP.

In order to differentiate between uptake of ³H-substance P into myelin, synaptosomes and mitochondria, crude mitochondrial P₂ fraction incubated with ³H-substance P (10⁻⁶ M) was further separated by means of a discontinuous sucrose density gradient according to the method of Gray and Whittaker (12). The results in Fig. 1 show that uptake of ³H-substance P was predominantly located in mitochondria. For comparison, crude mitochondrial P₂ fraction from rabbit spinal cord was incubated with ¹⁴C-glycine (10⁻⁶ M), a candidate for inhibitory transmitter in the spinal cord. As is shown in Fig. 1, the highest concentration was found in synaptosomes.

FIG. 1. Uptake of ³H-substance P and ¹⁴C-glycine into myelin (A), synaptosomes (B) and mitochondria (C) from rabbit diencephalon and spinal cord. Crude mitochondrial P₂ fraction from diencephalon and spinal cord was incubated for 10 min at 37°C with ³H-substance P (10⁻⁶ M) and ¹⁴C-glycine (10⁻⁶ M), respectively. Subfractions were then prepared according to the discontinuous sucrose density gradient method of Gray and Whittaker (12).

$$RSA = \frac{\% \text{ cpm (37°C-4°C)}}{\% \text{ protein}}$$

It is improbable that during a 10 min incubation a great deal of metabolism of ³H-substance P took place because Benuck and Marks (3) demonstrated that when substance P was incubated with 100,000 × g supernatant of rat brain homogenates only a small amount of arginine, phenylalanine, leucine and methionine was released during 10 min.

In summary, uptake of substance P into crude mitochondrial P₂ fraction differs from that of known putative transmitters in a number of ways: 1) The amount of substance P taken up into the fraction at 37°C was almost the same as that taken up at 0°C. 2) The pellet/medium ratio, calculated from the net uptake at 37°C scarcely exceeded 1. 3) The uptake took place predominantly in mitochondria, not in synaptosomes.

These results suggest that the uptake is passive diffusion or non-specific binding rather than the high affinity transport. Further studies are underway to determine whether similar results can be obtained in small regions of the brain or spinal cord that are enriched in substance P.

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PROTECTIVE EFFECT OF SODIUM POLYACRYLATE ON INDUCED DUODENAL ULCERATION IN GUINEA PIGS

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Sodium polyacrylate (PAS) which is used as a food additive is a high molecular polyanion and its water solution is highly viscous. We reported previously that PAS protected the experimental gastric ulceration in rats (1, 2) and have pointed out in reports that the agent has several promising properties regarding the prevention and treatment of gastric ulcerations. The present study was undertaken to evaluate the inhibitory effect of PAS against the experimental duodenal ulceration in guinea pig. Aluminium Silicate (Al. Si), an antacid, was employed as a positive control drug.

Duodenal ulceration was produced in guinea pigs by a modification of the method of Anderson and Watt (3). Guinea pigs, weighing 400–500 gm, were fasted for 24 hr, but allowed water *ad libitum*. Six aqueous solutions containing PAS or Al.Si, 1.25, 2.5 and 5.0 g/dl were prepared. Ten ml of the solution was given intragastrically once a day for two days. The control group was given the same volume of saline. On days 1 and 2 each animal was given promethazine hydrochloride 10 mg/kg (s.c.) one hr after the administration of the test substance, followed by a dose of histamine (free base) 10 mg/kg s.c. suspended in beeswax and mineral oil with a interval of 30 min. The animals were sacrificed 24 hr after the 2nd histamine injection. The duodenum was removed and the degree of damage was assessed numerically. The severity of the lesion was classified into three gradations: small ulcer (less than 3 mm in diameter), large ulcer (more than 3 mm) and perforated ulcer.