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A COMPARATIVE STUDY OF SUBSTANCE P FROM INTESTINE AND BRAIN

BY

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In 1931 Euler and Gaddum extracted a smooth muscle stimulating factor—substance P—from intestinal muscle and brain tissue. As the active principles from these two sources had the same biological effect on different smooth muscle preparations, and the same thermostability and solubility in different solvents, they have been regarded as identical (Bjurstedt, Euler, and Gernandt, 1940; Pernow, 1953). This assumption, however, has never been subjected to closer investigation. Pernow (1953) purified the substance from intestine (PI) and that from brain (PB) separately by adsorption on aluminium oxide and partition chromatography on cellulose. In all steps of the purification the two principles behaved similarly, and the most purified preparations gave the same R_P-value on paper chromatograms using *n*-butanol/acetic acid/water (40:10:50) as solvent.

Since highly purified preparations of both PI and PB are now available, it seemed worth while to make a more detailed chemical and biological study of the two principles. The results of these experiments are presented in this paper.

METHODS

Extraction and Purification.—Substances PI and PB were obtained from cattle by boiling the minced tissue in water at *p*H 4 and precipitation after filtration with ammonium sulphate according to Euler (1942). Each substance was further purified by adsorption on aluminium oxide columns according to a method previously described (Pernow, 1953). The activity of the preparations was about 200 units/mg.

Counter-current Distribution was performed in a 25-tube steel Craig apparatus (Craig and Post, 1949), using *n*-butanol/acetic acid/water (40:10:50) as distribution phases, 8 ml. for each phase. The substances were studied separately. The dry weight of material was kept constant in all experiments, which were carried out in the following way: 1,000 units of PB in 1 ml. distilled water was inactivated with 0.05 mg. crystalline chymotrypsin in Tyrode solution (*p*H 8.3) at 38° C. overnight. After checking that all PB activity was destroyed, the enzyme was inactivated by boiling. The solution was then concentrated to dryness *in vacuo* and the residue dissolved in the lower phase of tube 0, which also contained 1,000 units of active PI. The distribution then started. In another experiment PI was inactivated in the same way and mixed with active PB. After a 25-tube run, 1 ml. of each lower phase was concentrated to dryness *in vacuo* at room temperature, dissolved in distilled water, and immediately tested on guinea-pig ileum.

Paper Electrophoresis was performed in the apparatus described by Kunkel and Tiselius (1951). Solutions of 0.05M-acetate buffer (*p*H 4.6–4.8), barbitone buffer (0.05M-barbitone sodium and 0.05M-HCl, *p*H 7.2–7.5), 0.05M-ammonium hydroxide-ammonium chloride buffer (*p*H 9.1–9.2), and glycine buffer (0.05M-NaOH, and 0.05M-glycine containing 2.68 g. NaCl/l., *p*H 10.1–11.7) were used. The voltage applied was 220 V D.C. After 2–5 hr. the paper (Whatman 20) was dried at room temperature. The localization of the active substances was done by cutting the paper into strips (2 × 0.5 cm.) and immersing each piece in an intestinal bath. In this way the activity was easily localized, since the substance, when present, was rapidly eluted from the paper and produced a slow contraction of the gut.

Paper Chromatography.—The paper used for the chromatograms was Grycksbo OB. The following solvents were used: *n*-butanol/acetic acid/water (40:10:50), *n*-butanol/ethanol/water (40:10:10), phenol/0.1N-HCl, pyridine/water (65:35). Substances PB and PI were chromatographed both separately and mixed during 15 hr. in a descending run at 23–25° C. The position of the substances on the filter paper was localized by the same method as described for the electrophoretic papers. This was successful only if the papers were run in an atmosphere of nitrogen, since the substances were inactivated during chromatography in air.

Enzymic Inactivation.—A crystalline chymotrypsin preparation, purchased at the Delta Chemical Works, Inc., N.Y., was used. To 40 units of PI and PB (0.20 mg.) separately in 1 ml. water was added 5 μg. chymotrypsin in 1 ml. solution, buffered to *p*H 8. The

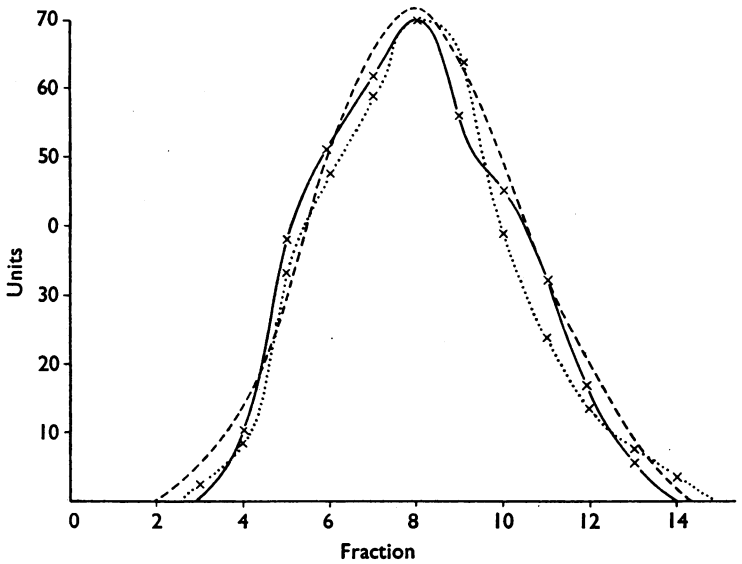

FIG. 1.—Counter-current distribution of substance P from intestine and brain. Solvent butanol/acetic acid/water (40:10:50). X——X, Substance P from brain; X···X, substance P from intestine; ----- K=0.5 (theoretical).

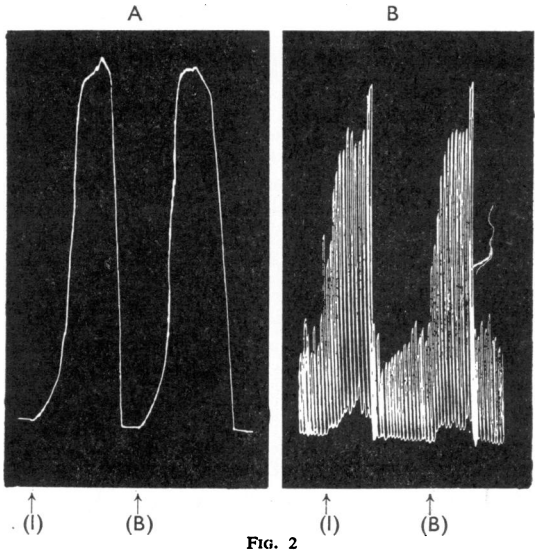

FIG. 2

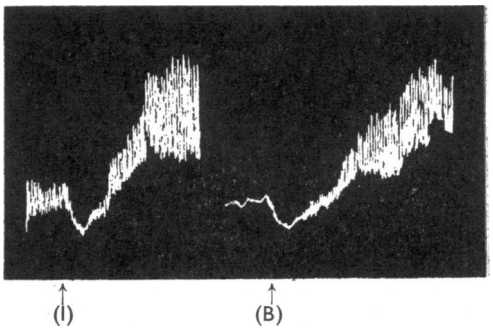

FIG. 3.—Rat duodenum. Effect of 2 units of substance P from intestine (I) and brain (B).

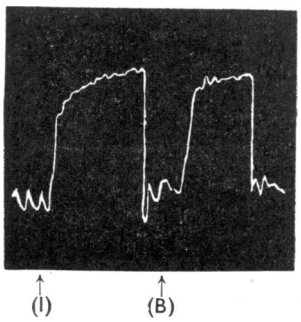

FIG 4

FIG. 2.—A, Isolated guinea-pig ileum. Bath volume 8 ml. Effect of 1 unit of substance P from intestine (I) and brain (B). B, Isolated rabbit jejunum. Bath volume 15 ml. Effect of 2 units of substance P from intestine (I) and brain (B).

FIG. 4.—Hen rectal caecum. Bath volume 15 ml. Effect of 1 unit of substance P from intestine (I) and brain (B).

FIG. 5.—Cat blood pressure. Effect of 40 units of substance P from intestine (I) and brain (B).

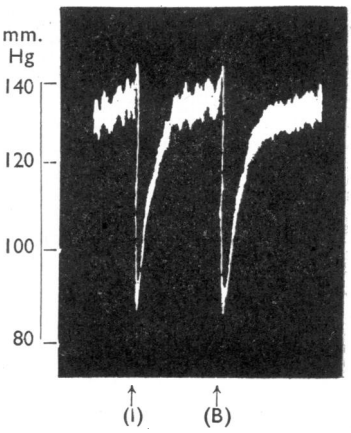

FIG. 5

incubation was performed at 38° C. It was always shown, in control experiments, that the gut was insensitive towards the pure enzyme.

Biological assay of substances PI and PB was on the isolated guinea-pig ileum, rabbit jejunum, rat duodenum, and hen rectal caecum, suspended in Tyrode solution. The guinea-pig and rat intestines were suspended in a 3 ml. bath, aerated with pure oxygen, and the rabbit and hen intestines in a 15 ml. bath aerated with 6.5% CO_2 in oxygen.

The vasodepressor actions of substances PI and PB were recorded from the carotid artery of rabbits anaesthetized with urethane (8 ml. 20% soln./kg., i.v.) and of cats anaesthetized with pentobarbitone sodium (35 mg./kg., i.p.).

Results

Counter-current Distribution.—Six experiments were performed, 3 with PB and 3 with PI in active form. In all distributions the greatest yield was obtained in tube 8, giving a distribution coefficient of 0.50 (Fig. 1). The yield of activity, about 40%, was the same for both substances.

Paper Electrophoresis.—Substances PI and PB were studied both separately and mixed. Within the *p*H-ranges used they showed the same mobility pattern. It was therefore not possible to separate them using this method.

Paper Chromatography.—Identical R_F values were obtained for substances PI and PB in the different solvents. For the purest preparations of both substances (2,000 u./mg.) these were 0.38 in *n*-butanol/acetic acid/water (40:10:50) and 0.92 in phenol saturated with 0.1N-HCl.

Enzymic Inactivation.—Earlier observations (Pernow, 1955) that substance P is rapidly inactivated with chymotrypsin were confirmed. Chymotrypsin (5 μg.) inactivated 40 units of PI and PB (0.25 mg.) in 30 min. No significant difference in the inactivation rate of PI and PB was found (Table I).

TABLE I

RATE OF INACTIVATION OF SUBSTANCE P FROM INTESTINE (PI) AND BRAIN (PB) BY CHYMOTRYPSIN

(40 units of substance P (0.20 mg.) incubated with 5 μg. of chymotrypsin at 38° C.)

Time (min.)	% Inactivation	
	PI	PB
1	0	0
3	25	30
8	40	40
12	60	65
16	70	75
21	85	
25		90
27	90	

Parallel Quantitative Biological Assays.—When substances PI and PB with the same degree of purity were tested on different isolated intestines the same height and type of contraction were always obtained. The ratio therefore was 1:1. The time course of the contraction (determined by measuring the time necessary for obtaining successive levels of contraction) was also the same for identical amounts of PI and PB (Figs. 2–4).

A fall in the blood pressure of about 20 mm. Hg was produced by 3–5 units in the rabbit and by 20–30 units in the cat. Identical effects on the blood-pressure recordings were obtained with equal amounts of PI and PB (Fig. 5).

Discussion

Before further investigation of the physiological actions of substances PI (from intestine) and PB (from brain) we thought it necessary to see if these substances were identical. Our comparative chemical and biological studies have given similar results for both, suggesting that the two substances are identical. Umrath (1953) found, however, that PI and PB were inactivated by extracts of nervous tissue at different rates: PB was destroyed by a proteolytic factor in nervous tissue 2–3 times faster than PI. Umrath's extracts of substance P, however, were prepared by boiling the tissue in a solution of 0.65% NaCl and 0.01% $CaCl_2$. It is known that such crude extracts also contain other smooth muscle stimulating substances such as 5-hydroxytryptamine and Darmstoff. The differences obtained by Umrath between PI and PB might therefore have been caused by the presence of such substances in varying proportions in the different extracts.

That the methods used in this paper are useful in separating substances with similar chemical and biological actions has recently been shown by Pernow and Rocha e Silva (1955) in a comparative study of bradykinin and substance P. These principles, both of which are polypeptides, inactivated by chymotrypsin, and both of which stimulate smooth muscle organs, could be completely separated by means of paper electrophoresis and counter-current distribution. A definite difference has also been demonstrated by comparing their actions on different isolated intestines.

Summary

1. Purified substance P preparations from cow's intestine and brain have been compared chemically and biologically.

2. It was not possible to distinguish chemically, physically, or biologically between substance P from intestine and that from brain.

3. It is therefore suggested that these substances are identical.

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