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An Endogenous Inhibitor of N-Methyltransferase Activity and Opiate Receptor Binding in Rabbit Tissue[1]

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ROSENGARTEN, H., G. MARZULLO AND A. J. FRIEDHOFF. *An endogenous inhibitor of N-methyltransferase activity and opiate receptor binding in rabbit tissue.* PHARMAC. BIOCHEM. BEHAV. 5: SUPPL. 1, 147–150, 1976. · We have characterized and purified a peptide extracted from newborn rabbit brain, lung and liver. This peptide has a molecular weight of 1500 and has the ability to inhibit adult rabbit lung N-methyltransferase activity in vitro and can also bind to opiate receptor in rat brain homogenate.

Peptide Inhibitor N-Methyltransferase inhibitor Opiate receptor

MANDEL and Morgan [4] and Saavedra and Axelrod [8] demonstrated that the activity of N-methyltransferase, the enzyme involved in the transformation of tryptamine or N-methyltryptamine to DMT could be increased by subjecting the tissue source to dialysis. On the basis of this they proposed that there was a dialysable inhibitor which prevents the reaction from occurring in vitro. We have characterized such an inhibitor and found it to be a peptide which is present in abundance in many tissues and in particularly high concentration in newborn rabbit brain, lung and liver [5,6]. No inhibitor of N-methyltransferase activity could be detected in adult rabbit lung in which N-methyltransferase activity was first described by Axelrod [1]. Adult rabbit lung N-methyltransferase activity was not affected by dialysis whereas dialysis increased N-methyltransferase activity in newborn rabbit tissues [6]. Because of the high concentration of inhibitor in the newborn, rabbit neonatal tissues were used for the purification and characterization of this inhibitor.

METHOD

For preparation of the inhibitor for the present study newborn rabbits were killed by decapitation. Brain, lung and liver tissues were homogenized in 2 volumes/weight of ice cold distilled water. Homogenate was heated in boiling water bath for 3 min and centrifuged at 100,000 × g for 30 min. The high-speed supernatant was dialysed for 2 hr in distilled water 1:10 volumes and lyophylized. Lyophilized dialysate was chromatographed on Sephadex G25 (medium grade). Fractions demonstrating N-methyltransferase inhibitory activity were located in the linear region of the elution curve. These fractions were pooled and rechromatographed on Sephadex G25 (fine grade). Molecular weight was estimated on a calibrated column of Sephadex G25, (fine grade) in 0.10M PO_4 buffer pH 7.0 using bacitracin mol. wt. 1450 (Schwartz/Mann), lys-vasopressin mol. wt. 1086 and oxytocin mol. wt. 1007 (Sigma). Void volume was determined with blue dextran.

RESULTS

The N-methyltransferase inhibitory activity tested versus adult rabbit lung enzyme was separated with little overlap, into two peaks (Fig. 1), the taller Peak I of approximate molecular weight 1500, and Peak II of approximate molecular weight 1300. The relative inhibitory potency of these two peaks varies with the preparation, and depends on the freshness of the preparation and the lyophylization time. Peak I is usually more potent, but upon standing or with increased lyophylization time the inhibitory activity of Peak I decreased while that of Peak II increased. Occasionally, a small third peak also formed. From these findings it appears that Peak I of mol. wt. 1500 represents the native form which cascades down to lower molecular weight forms retaining some of the inhibitory activity (Fig. 2).

Table 1 is a summary of the results of the purification of material in Peak I from Sephadex G25. Dialysis of the boiled 100,000 × g supernate resulted in a 38.7 fold purification of the inhibitor. A further 894 fold purification was achieved by chromatography on Sephadex G25, medium. Rechromatography on Sephadex G25, (fine), resulted in an additional 6.29 fold purification, for a total purification of 2175 fold. Purified inhibitor was subjected to digestion by proteolytic enzymes and degradation by heating. Material from both peaks was resistant to heating at 100° C for 10 min, but heating at 96° C for several hours resulted in complete loss of activity. Incubation of purified material with trypsin resulted in digestion of both forms of the inhibitor. Little activity was detectable in the original fractions after the inhibitor was digested by trypsin and

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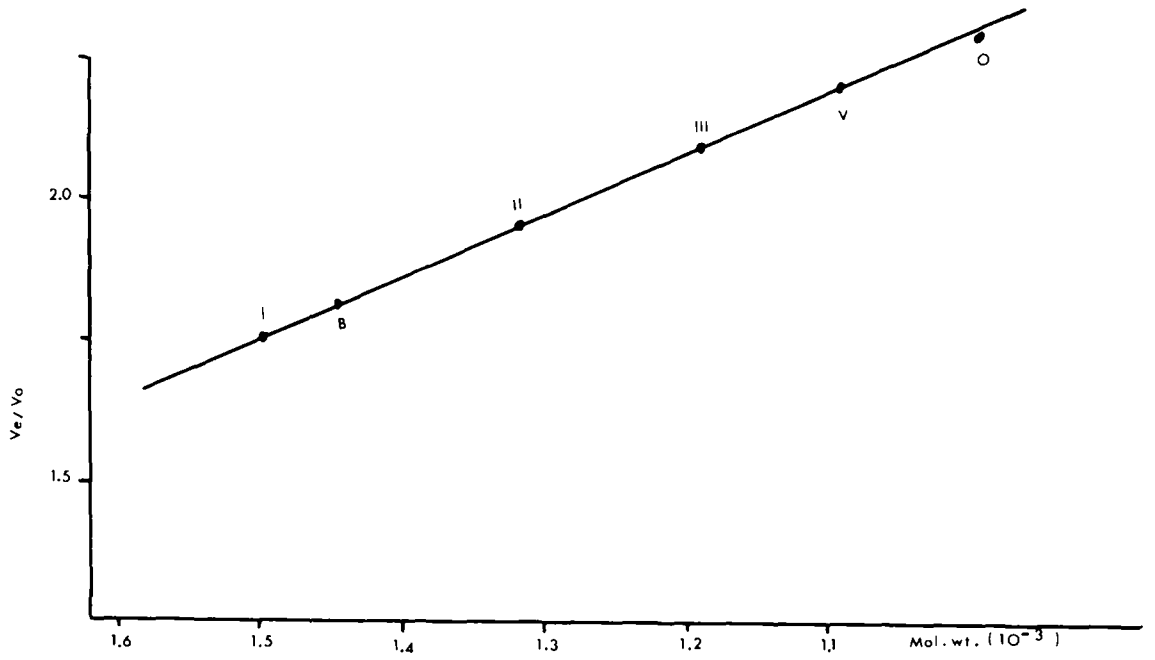

FIG. 1. Estimation of molecular weights of inhibitors I and II on sephadex G-25 (fine grade); B-Bacitrcin, V-Vasopressin, O-Oxytocin.

TABLE 1

SUMMARY FOR THE PURIFICATION SCHEME FOR INHIBITOR I

Stage of Purification	EC_{50} μg protein/ml	Relative Activity
100,000g super	2110	1
Dialysis	54.5	38.7
Sephadex G-25 (medium)	6.1	346
Sephadex G-25 (Fine)	0.97	2175

The specific activity is expressed as the number of μg of protein in a sample producing 50% inhibition of N-methyltransferase activity.

then rechromatographed on Sephadex G25, fine grade. Pooled fractions from Peak II, incubated with carboxypeptidase A (CPA), showed partial loss of activity. Leucine aminopeptidase was not effective against either substance.

Several authors have reported that certain brain peptides bind to the so called opiate receptor [2, 3, 7, 9, 11, 12, 13]. The two peptides described here and which we also described in an earlier publication as specific inhibitors of N-methyltransferase activity [5,6] were tested for their ability to inhibit stereospecific binding of ^{3}H-etorphine to opiate receptors in rat brain according to Simon *et al.* [10]. We found that pooled fractions of material from both peptide peaks, resulting from purification of material from newborn rabbit brain, lung and liver, could inhibit stereospecific opiate receptor binding, and that the properties of fractions from Peaks I and II are the same as these described for the so called endogenous ligand of opiate receptors.

Figure 2 shows the overlap of the N-methyltransferase inhibitory activity of Peak I from rabbit brain and its inhibition of opiate receptor binding. Active fractions produced over 75% inhibition of opiate binding. Figure 3 shows inhibition of N-methyltransferase activity and opiate binding by Inhibitor I from lung tissue. Peak I derived from rabbit liver also showed the same ability to inhibit both N-methyltransferase activity and opiate binding (Fig. 4). This is consistent with the properties of the previously described endogenous ligand for opiate receptors which was previously reported to be present only in brain [3, 7, 9, 11, 12] or pituitary [2,13]. The sensitivity of our inhibitor to degradation by proteolytic enzymes and their molecular weights of 1500 and 1300, respectively, are consistent with these compounds being of peptidic nature.

DISCUSSION

Our peptide seems to be different from previously described peptides. Goldstein's group [2,13] describes a peptide present only in pituitary extract, which mimics morphine activity and has a higher molecular weight of 1750. Snyder's group [7,9] describes two pentapeptides of lower molecular weight present in synaptic vesicles, which are sensitive to degradation by carboxypeptidase A and B and leucine aminopeptidase but not trypsin. Our peptide is present in the soluble fraction of newborn rabbit brain, lung and liver and has a different molecular weight and derives from different tissues than those described by either the Goldstein [2,13] or Snyder [7,9] groups. Our peptide inhibitor is sensitive to trypsin digestion, but CPA only slightly modifies its activity. The pentapeptide, enkephalin, described by Hughes [3] was not present in our preparation. Fractions eluted from G25, (fine) in the elution location of synthetic enkephalin do not retain the ability to inhibit binding of an opiate agonist to opiate receptors.

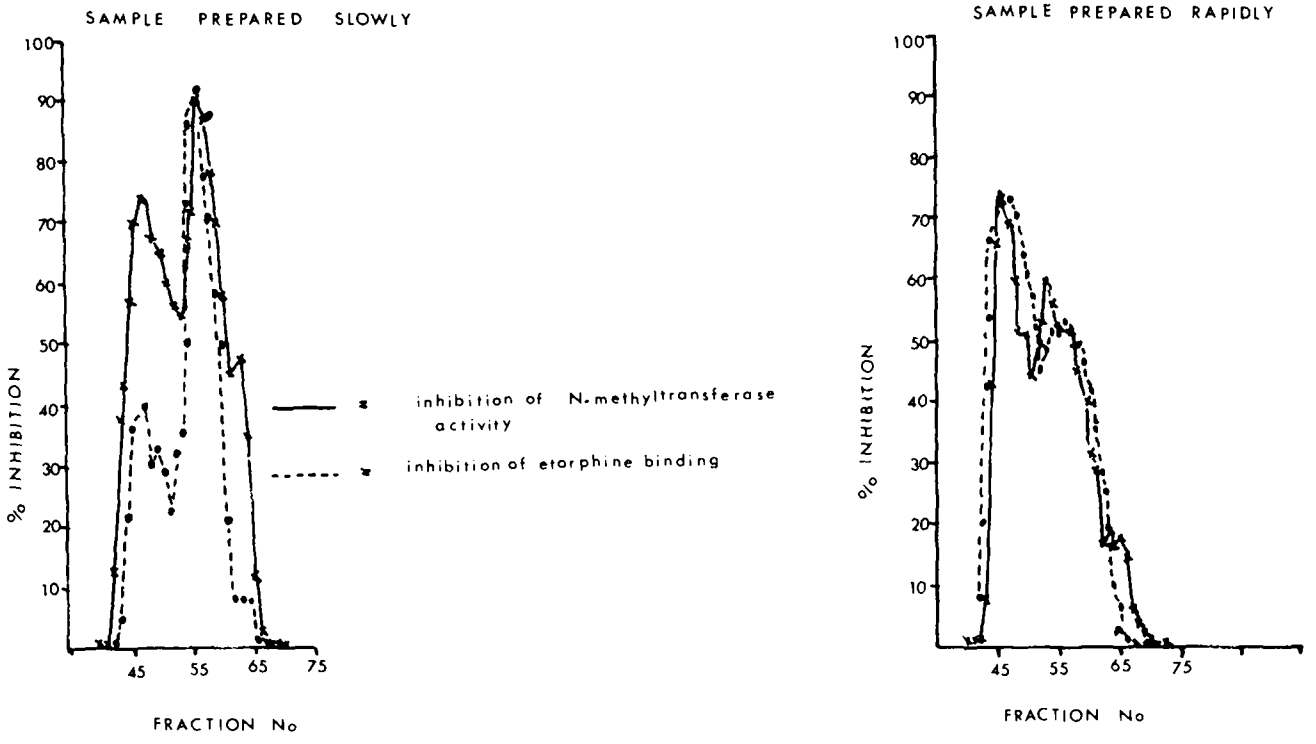

FIG. 2. Chromatography of brain peptide on sephadex G-25 (fine grade).

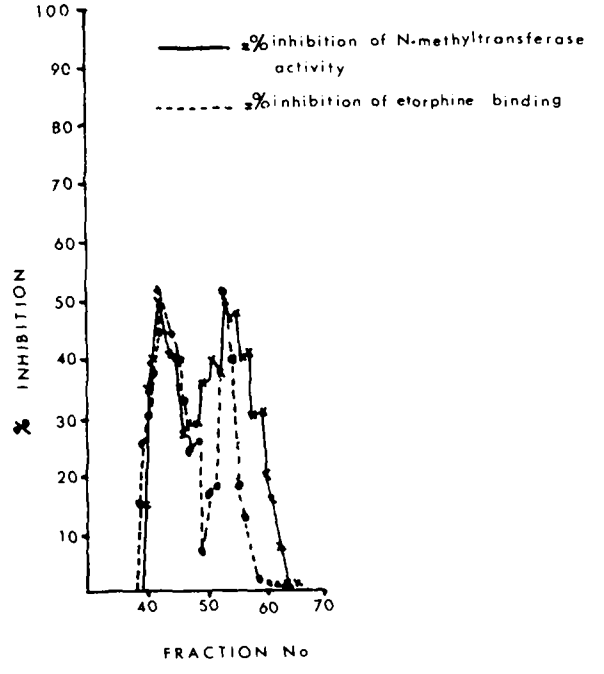

FIG. 3. Chromatography of lung peptide on sephadex G-25 (fine).

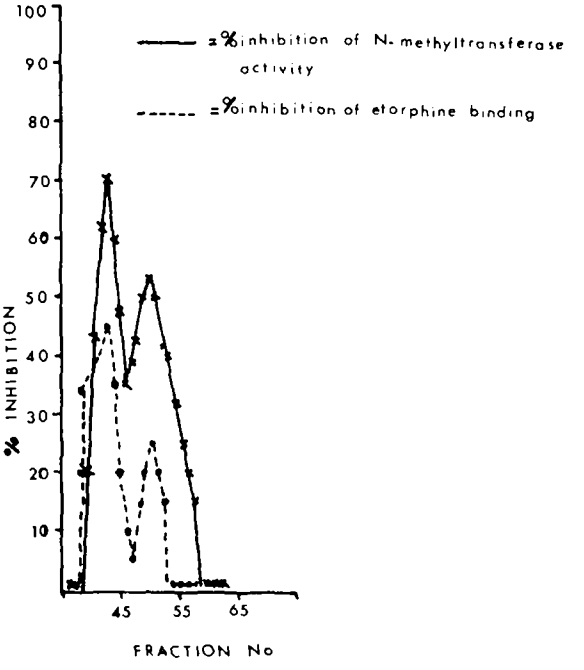

FIG. 4. Chromatography of liver peptide on sephadex G-25 (fine).

We have isolated a substance from rabbit brain, liver and lung which is capable of inhibiting N-methyltransferase activity. This substance has the properties of a small peptide.

ACKNOWLEDGEMENT

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