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Binding of potassium [^{3}H]embelate to rat brain synaptosomes

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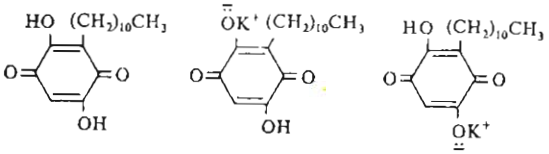
ABSTRACT [^{3}H]Embelate (potassium salt of 2,5-dihydroxy-3-undecyl-1,4-benzoquinone), a new analgesic compound, showed a specific and saturable binding in rat brain synaptosomes which was not influenced by naloxone and morphine. The binding characteristics were correlated with its non-narcotic central analgesic action.

KEY WORDS potassium embelate; analgesics; synaptosomes; competitive binding

Embelin (2,5-dihydroxy-3-undecyl-1, 4-benzoquinone) is found in appreciable quantity in the fruits of *Myrrsine americana, Embelia ribes, Embelia robusta* and other *Embelia spp*[1]. Potassium embelate (PE) showed a potent non-narcotic analgesic action when administered by po, im or icv routes to animals[2]. Pharmacokinetic studies suggested that PE readily crossed blood-brain barrier. Both iv and po administrations of PE to rats produced measurable concentrations of PE in brain within 30 min[3]. The present study was carried out on the basis of an assumption that there might be a binding site for PE in the CNS. This paper deals with *in vitro* binding of [^{3}H]PE to rat brain synaptosomes and possible correlation of binding characteristics with its pharmacological activity.



embelin and its potassium salts

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MATERIALS AND METHODS

Embelin was isolated from *Embelia ribes* and its potassium salt was prepared by the method reported previously[2,3]. [^{3}H]PE was of 95% rediochemical purity. Morphine sulphate was obtained from Sigma Chemical Co. USA, and naloxone-HCl from Endo Laboratories, USA. All other chemicals were reagent grade.

Charles-Foster rats (100-175 g) kept under uniform husbandry conditions were used. Synaptosomes were obtained by a rapid method[4]. The samples (synaptosome pellets) were resuspended in sodium phosphate buffer 6.0 mmol · L^{-1} containing NaCl 0.2 mol · L^{-1} (pH 7.0), and diluted to 5.0 mg protein / ml with this buffer for routine use in binding assay. Protein content was determined by the colorimetric method[5]. Binding assay was carried out by incubating 500 μl of the sample in triplicate at 30℃ for 30 min. The sample contained [^{3}H]PE approximately 80 μmol · L^{-1} in the absence or presence of unlabeled PE or other compounds. The reaction was stopped by centrifugation at 48 000 × g for 10 min. The pellet was gently washed twice with the buffer and dissolved in 5.0 ml of dioxane-based scintillation fluid. After the disappearence of chemiluminescence, the radioactivity was measured in Beckman LS counter (3150 P). Specific [^{3}H]PE binding was determined by substracting the nonspecific binding (in presence of unlabeled PE 1.0 mmol · L^{-1}) from the total binding.

RESULTS

As shown in Fig 1 the amount of [^{3}H]PE bound to the synaptosome preparation

gradually decreased with the increasing concentration of unlabeled PE in the incubating medium. This effect was seen only upto a concentration of 1.0 mmol · L^{-1} of unlabeled PE. This showed that [^{3}H]PE bound to a specific binding site was gradually displaced by unlabeled PE, which finally saturated the site. The difference between the amounts of bound [^{3}H]PE in the absence and presence of excess unlabeled PE (1.0 mmol · L^{-1}) can be regarded as the amount of specifically bound PE.

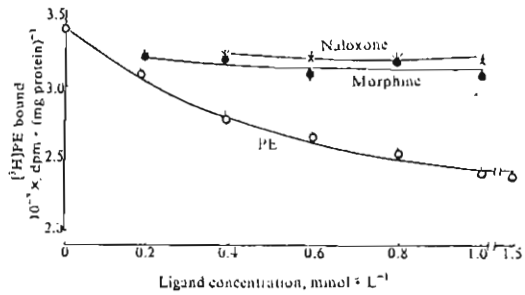

Fig 1. Displacement of [^{3}H]PE bound to synaptosomes by unlabeled PE. n=6–8. $\bar{x}\pm$ SD.

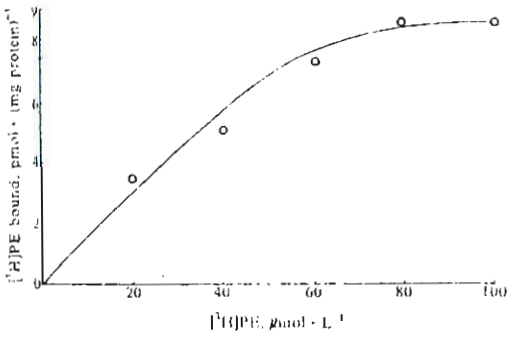

Fig 2. Specific binding of [^{3}H]PE to synaptosomes from rat brain as a function of [^{3}H]PE concentration. n=6–8. $\bar{x}\pm$ SD.

The amount of specifically bound [^{3}H]PE was increased with an increase of theconcentration of [^{3}H]PE (Fig 2), and the PE binding seemed to be a saturable process, with half maximal binding at 30 μmol · L^{-1}. The apparent equilibrium dissociation constant (K_d) and the quantity of the PE bound at saturation (B_{max}) were estimated from the double reciprocal plot of the data to be about 62.5 μmol · L^{-1} and 13.5 pmol · mg^{-1}protein, respectively (Fig 3).

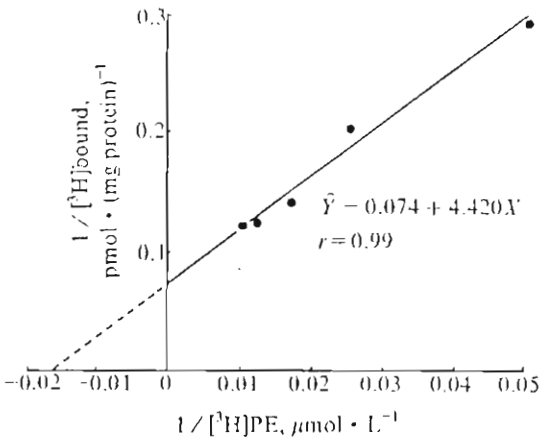

Fig 3. Double reciprocal plot of specific [^{3}H]PE binding to synaptosomes.

The competition of [^{3}H]PE binding by morphine and naloxone are shown in Fig 1. These substances had no effect on the specific binding.

The optimal temperature for the specific [^{3}H]PE binding was approximately 30℃ (Fig 4) and optimal incubation time 30 min (Fig 5).

The effect of increasing protein (synaptosome) concentration on the [^{3}H]PE binding is summarised in Tab 1. It was observed that the binding was increased significantly upto a protein concentration of 3 mg · ml^{-1} in the incubation medium. Further increase (4–5 mg protein / ml of incubation medium) did not increase any more. This amount of pellet protein may be considered to be saturating concentration.

Tab 2 shows the effect of repeated washed

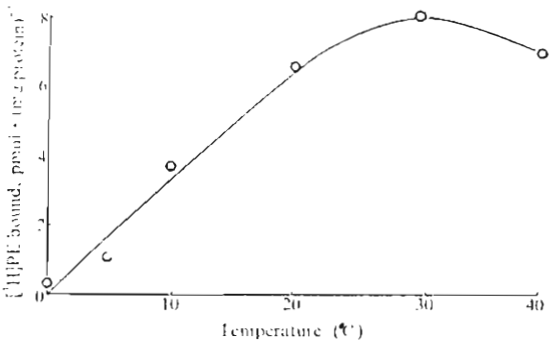

Fig 4. Effect of temperature on specific [^{3}H]PE binding to synaptosomes. $n=6$–8. $\bar{x}\pm$ SD.

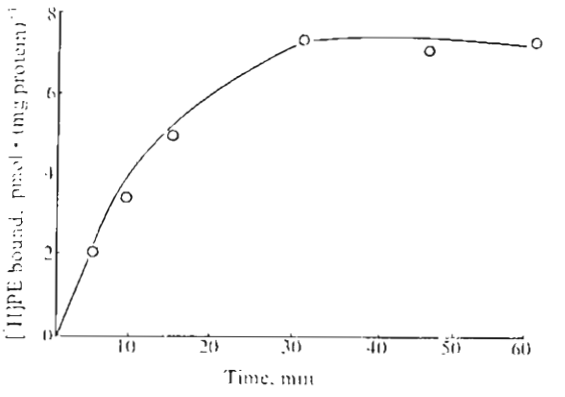

Fig 5. Time-dependent binding of [^{3}H]PE to synaptosomes. $n=6$–8. $\bar{x}\pm$ SD.

Tab 1. Effect of protein of synaptosome pellet on [^{3}H]PE binding. $n=5$–7, $\bar{x}\pm$ SD.

protein (mg / assay)	[^{3}H]PE bound (pmol / mg protein)
1.0	2.5 ± 0.6
2.0	5.0 ± 1.7
3.0	9.0 ± 1.8
4.0	10.7 ± 2.9
5.0	12.0 ± 2.9
6.0	11.9 ± 2.0
8.0	12.0 ± 2.2

of pellets on synaptosome bound [^{3}H]PE. About 19% of radioactivity in the initial pellet was removed by a single wash and 21% after the second wash. After multiple washes no appreciable changes were noted. It showed that most of the radioactivity was in pellet, not in solvent.

Tab 2. Loss of radioactivity in synaptosome pellet before (A) and after (B) the wash. $n=3$–6, $\bar{x}\pm$ SD.

Number of washes	$\frac{A-B}{A} \times 100\%$
1	19.0 ± 2.1
2	21.1 ± 2.5
3	22.0 ± 1.9
4	21.9 ± 2.2
5	21.7 ± 2.5

DISCUSSION

It was demonstrated that sites for [^{3}H]PE binding were specific, saturable and dissociable. The binding amount was dependent on synaptosome protein concentration. Earlier. *in vivo* studies[3] revealed high concentrations of [^{3}H]PE in rat brain between 30 min and 2 h after administration. The amount of bound [^{3}H]PE was found to be 17 nmol • mg^{-1} synaptosome protein (data not shown). In the present work the concentration of [^{3}H]PE used for sustitutive binding experiments was 80 μmol • L^{-1} which was equivalent nearly to 16 nmol • mg^{-1} synaptosome protein. This concentration is different from the K_d of [^{3}H]PE (62.5 μmol • L^{-1}) which was somewhat lower than that expected from its pharmacokinetic data, but corresponded approximately to its amount *in vivo*. Morphine and naloxone were unable to displace appreciably PE binding, suggesting that the binding sites for PE were not similar to those affected by narcotic agonists and antagonists. This observation would reinforce the earlier finding that PE possessed central and non-narcotic properties.

Synaptosomes primarily store and metabolize acetylcholine, which could possibly be involved in PE-induced analgesia in rats.

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Effects of estradiol on carbonic anhydrase and Mg^{2+}-HCO_3^--ATPase activities in rat duodenal microvilli and kidney tubules

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ABSTRACT The cytosol carbonic anhydrase (CA) and microvillus membrane Mg^{2+}-dependent, HCO_3^--stimulated ATPase (Mg^{2+}-HCO_3^--ATPase) activities implicated in ion transport were determined in duodenal mucosa and renal tubule of ovariectomized and estradiol (E_2)-treated ovariectomized rats. CA and Mg^{2+}-HCO_3^--ATPase activities in duodenum remained unchanged after ovariectomy, and sc E_2 200 $\mu g \cdot d^{-1} \times 7$ d decreased the activity of CA. Both the enzymes in kidney exhibited a similar sensitivity to ovariectomy, and the lowered activity of Mg^{2+}-HCO_3^--ATPase following ovariectomy was restored to near normal after administration of E_2. These results suggest that E_2 may be a factor in regulation of the above enzymes from the duodenum and kidney of rats, the regulation of E_2 on these 2 enzymes in rat duodenum is greatly different from that in rat kidney.

KEY WORDS estradiol; carbonic anhydrase; magnesium adenosine triphosphatase; microvilli; duodenum; kidney tubules

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Estrogen receptors have been described in a number of nonreproductive target tissues, including kidney, liver, gut, heart and brain. The induction of estrogen on some enzyme systems, for example, 16-hydroxysteroid dehydrogenase, carbonic anhydrase and Na^+-K^+-ATPase was reported in various tissues. CA in the cytosol and Mg^{2+}-HCO_3^--ATPase in the brush border membrane of duodenal mucosa and renal tubule are thought to be related to several ionic transports across the membrane, yet little is known of the biochemical effect of estrogen on these enzymes in duodenum and kidney. In this paper, both CA and Mg^{2+}-HCO_3^--ATPase activities of duodenal mucosa and renal tubule from ovariectomized and E_2-treated ovariectomized rats were examined to clarify the effect of estrogen in the enzyme level.

MATERIALS AND METHODS

Rats Wistar ♀ rats, weighing 196 ± SD 10 g, were used. Bilateral ovariectomies