MOLECULAR PHARMACOLOGY, 15, 751-753

SHORT COMMUNICATION

Stereospecific Opiate Binding in Bovine Adrenal Medulla

CHARLES CHAVKIN, B. M. COX, AND AVRAM GOLDSTEIN

Stanford University and Addiction Research Foundation, 701 Welch Road, Palo Alto, California 94304

(Received October 27, 1978)
(Accepted December 16, 1978)

SUMMARY

CHAVKIN, CHARLES, COX, B. M. & GOLDSTEIN, AVRAM. (1979) Stereospecific opiate binding in bovine adrenal medulla. Mol. Pharmacol., **15**, 751-753.

In this report we describe a population of high affinity, saturable, opiate binding sites in the adrenal medulla. These sites show the typical sensitivity to sodium ions characteristic of opiate binding sites found in brain tissue. No comparable high affinity sites were detectable in adrenal cortex.

In the course of the search for peripheral sites of pituitary β-endorphin action, we chose to examine the adrenal medulla and adrenal cortex for opiate receptors. Adrenal tissue homogenates were prepared essentially as described for brain opiate receptor studies (1, 2). Fresh bovine adrenal glands were collected within 5 min of slaughter, frozen immediately on dry ice, and stored at −70° until use. Adrenal cortex and medulla were dissected apart, then separately minced with a Tissuemizer D18K for 10 sec before homogenizing in a motor driven, glass-Teflon homogenizer containing 10 vol of ice-cold 100 mM Tris-HCl buffer (pH 7.4). Crude membrane fractions were prepared by first sieving the homogenate through nylon mesh, then pelleting the effluent by centrifugation at 17,000 × g for 20 min at 2° in a Sorvall SS34 rotor. Membrane pellets were washed once with 40 vol cold Tris buffer and once with glass distilled water, then resuspended in Tris buffer at a concentration of 20% (w/v) based on original tissue weight.

Binding of the opiate antagonist ^{3}H-naltrexone to the membrane suspension was determined using radioreceptor assay technique as described by Medzihradsky (3). Stereospecific binding is defined as the difference between the ^{3}H-naltrexone binding to the membrane preparation in the presence of excess dextrallorphan (the inactive stereoisomer of the opiate antagonist levallorphan) and that measured in the presence of excess levallorphan (4). In a typical experiment, 200 µl of either 250 mM KCl or NaCl in 100 mM Tris-HCl buffer (pH 7.4) were added to 200 µl of a 20% membrane suspension in Tris buffer followed by 50 µl of either 12.5 µM dextrallorphan (Hoffmann-La Roche) or 12.5 µM levallorphan tartrate (Hoffmann-La Roche). Next 50 µl of the appropriate concentration of ^{3}H-naltrexone (Research Triangle Institute, 6.4 Ci/mmole) were added, and the assay tubes were incubated for 20 min at 23°. Bound radioactivity was separated from free by filtration through glass fiber filters (Whatman GF/C), and the bound counts were measured by liquid scintillation counting of the dried filters. At less than saturating concentrations, more than 80% of total binding to adrenal medulla membranes was stereospecific.

Scatchard analysis of a representative

This investigation was supported by United States Public Health Service Grant DA-1199 and National Institute of Health Training Grant GM-01749.

0026-895X/79/030751-03$02.00/0

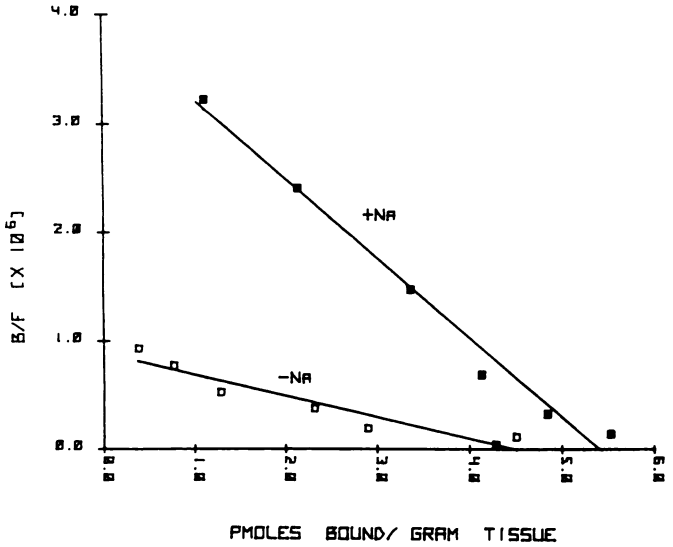

FIG. 1. *Scatchard plot of ^{3}H-naltrexone stereospecific binding to 8% bovine adrenal medulla membranes in the presence of 100 mM KCl, 100 mM Tris-HCl pH 7.4 (-Na) and 100 mM NaCl, 100 mM Tris-HCl pH 7.4 (+Na)*

B/F is the ratio of moles ^{3}H-naltrexone stereospecifically bound/gram tissue to concentration of unbound ^{3}H-naltrexone in the assay. Each point is the mean of duplicate determinations. The lines are drawn by least squares linear regression analysis.

bovine adrenal medulla membrane binding experiment is shown in Fig. 1. The dissociation constant for the specific ^{3}H-naltrexone binding sites is 1.53 ± 0.41 nM in the presence of Na^+ and 3.93 ± 0.84 nM in K^+ buffer (mean ± SEM for the three determinations). This increase is characteristic of the opiate receptor binding of narcotic antagonists (1, 5). Also, the affinity values and the magnitude of the Na^+ effect are comparable to that observed in ^{3}H-naltrexone binding studies of opiate receptors in brain tissue (Dr. C. E. Dunlap III, personal communication). Site density is estimated as 4.57 ± 0.52 pmol/g tissue in the presence of Na^+, 25% of that seen in guinea pig brain homogenate (minus cerebellum) under the same assay conditions (data not shown).

Since enkephalin immunoreactivity has been described in human adrenal medullary tumors (6) and in rabbit and rat sympathetic ganglia (7), it is possible that the binding sites demonstrated here mediate the effects of local enkephalin release. However, a physiologic role has not as yet been elucidated.

The derivation of β-endorphin and ACTH from a common precursor (8) and the suggested concomitant release from the rat pituitary (9) suggest that these hormones might also share a common target in the adrenal cortex. Low levels of specific ^{3}H-naltrexone binding to bovine adrenal cortex were seen at ^{3}H-naltrexone concentrations greater than 10 mM; however, less than 10% of the total binding was stereospecific. At the concentration of ^{3}H-naltrexone givine half saturation of the adrenal medulla binding sites (5 nM), cortex membranes stereospecifically bound 0.12 pmole/g tissue, only one-sixth of that bound to the adrenal medulla under the same conditions. The adrenal cortical membrane binding sites were not saturable at concentrations of ^{3}H-naltrexone below 10 μM indicating that these sites are of low affinity ($K_d > 10^{-6}$ M). Bovine plasma levels of β-endorphin have not been reported, but in view of the very low levels in rat plasma (0.4–2.6 nM) (9) and even lower levels in human plasma (about 10–30 pM) (10), it is extremely unlikely that these low-affinity binding sites in adrenal cortex mediate effects of circulating β-endorphin.

REFERENCES

1. Pert, C. B., Pasternak, G. & Snyder, S. H. (1973) Opiate agonists and antagonists discriminated by receptor binding in brain. *Science* **182,** 1354–1361.
2. Simon, E. J., Hiller, J. M. & Edelman, I. (1973) Stereospecific binding of the potent narcotic analgesis [^{3}H] etorphine to rat brain homogenate. *Proc. Natl. Acad. Sci. (USA)* **70,** 1947–1949.
3. Medzihradsky, F. (1976) Stereospecific binding of etorphine in isolated neural crest cells and in retina, determined by a sensitive microassay. *Brain Research* **108,** 212–219.
4. Goldstein, A., Lowney, L. I. & Pal, B. K. (1971) Stereospecific and nonspecific interactions of the morphine congener levorphanol in subcellular fractions of mouse brain. *Proc. Natl. Acad. Sci. (USA)* **68,** 1742–1747.
5. Simon, E. J., Hiller, J. M., Groth, S., & Edelman, I. (1975) Further properties of stereospecific opiate binding sites in rat brain: on the nature of the sodium effect. *J. Pharmacol. Exp. Ther.* **192,** 531–537.
6. Sullivan, S. N., Bloom, S. R. & Polak, J. M. (1978) Enkephalin in peripheral neuroendocrine tumours. *Lancet* **1,** 986–987.
7. Di Guilio, A. M., Yang, H. Y. T., Lutold, B. E., Fratta, W. & Costa, E. (1978) On the enkephalin content of sympathetic ganglia. *The Pharmacologist* **20,** 167.
8. Mains, R. E., Eipper, B. A. & Ling, N. (1977) Common Precursor to corticotropins and endorphins. *Proc. Natl. Acad. Sci. (USA)* **74,** 3014–3018.
9. Guillemin, R., Vargo, T., Rossier, J., Minidi, S., Ling, N., Rivier, C., Vale, W. & Bloom, F. (1977) β-endorphin and adrenocorticotrophin are secreted concomitantly by the pituitary gland. *Science* **197,** 1367–1369.
10. Ghazarossian, V., Dent, R. R., Ross, M., Cox, B. M. & Goldstein, A. (1978) Radioimmunoassay for β-endorphin in human plasma. In: *Characteristics and Functions of Opioids, Developments in Neuroscience Vol. 4,* (J. M. Van Rhee and L. Terenius, eds.), Elsevier North Holland, 273–274.