Biochemical Pharmacology, Vol. 23, pp. 2349-2352. Pergamon Press, 1974. Printed in Great Britain.

EFFECT OF α-ADRENERGIC BLOCKERS ON NALOXONE-BINDING IN BRAIN

Theodore J. Cicero, Carol E. Wilcox and Edward R. Meyer

Department of Psychiatry, Washington University

School of Medicine, St. Louis, Missouri, 63110

(Received 13 May 1974; accepted 20 May 1974)

Communicated by: Robert H. Roth

In several recent studies, we have shown that α-adrenergic blocking agents possess a degree of antinociceptive activity themselves, markedly increase morphine's analgetic and toxic effects, and effectively suppress the expression of the narcotic withdrawal syndrome in the rat (1,2,3). Since β-adrenergic blocking agents and numerous other agents possessed none of these properties, these data suggest that the narcotics and α-adrenergic blockers may be closely related. However, since all of the data up to the present are indirect, we cannot rule out the possibility that the interaction we have observed between these two classes of drugs merely represents the summation of two totally independent processes. The purpose of the studies described in this paper was to obtain more direct evidence of an interaction between morphine and α-adrenergic blocking agents. Pert and Synder (4,5) previously have demonstrated that stereospecific binding of naloxone occurs in rat brain homogenates and that various narcotic agonists and antagonists effectively reduce naloxone-binding. We have employed this preparation to examine whether α-adrenergic blockers would displace naloxone from the so-called "opiate receptor."

Methods

Rat brains were homogenized in 0.05M Tris-HCl buffer (pH = 7.4). All drugs

were dissolved in the Tris buffer. The standard incubation mixture consisted of 20 mg (wet-weight) tissue in a total volume of 2 ml of Tris buffer. After the addition of drug, the samples were incubated for 10 min at 37°C in a shaking water bath. They were removed to an ice water bath, ^{3}H-naloxone was added (final concentration of 5 X 10^{-9}M) and the samples were incubated for an additional 15 min. Two "blanks" were included in all experiments, a tissue blank, which was identical to the standard incubation mixture except that no ^{3}H-naloxone was added, and a levorphanol blank. The levorphanol blank consisted of 20 mg of tissue in 2 ml Tris to which levorphanol (1 X 10^{-7}M) was added during th 10 min incubation period prior to the addition of ^{3}H-naloxone. The purpose for including this blank was to provide a measure of the degree of non-specific binding of naloxone to rat brain homogenates as described previously by Pert and Snyder (4,5). The levorphanol blank was subtracted from all sample values to yield a measure of the "specific tissue binding" of naloxone. A typical experiment, thus consisted of a tissue blank, a levorphanol blank and a series of sample tubes in which naloxone-binding was examined in the absence or presence of various drugs. Following incubation with naloxone, the samples were filtered through Whatman GF/A glass fiber circles and the filters counted as described perviously (4,5).

All drugs evaluated in these studies were screened initially at 1 X 10^{-5}M to determine whether inhibition of bound naloxone occurred. If a drug produced greater than 15% inhibition of naloxone-binding, a more thorough examination was conducted to determine the drug concentration producing a 50% inhibition of specific naloxone tissue binding.

Results

The effects of various drugs on the stereospecific binding of naloxone to rat brain homogenates is shown in Table 1. In agreement with previous studies (4,5) narcotic agents significantly inhibited the specific binding of naloxone. Of particular interest, however, was that α-adrenergic blocking compounds also significantly inhibited the specific binding of naloxone to brain. In terms of

the relative effectiveness of the α-blockers in inhibiting naloxone-binding, these agents were comparable to the narcotic drugs methadone and codeine.

Table 1

The effects of various drugs on the specific tissue binding of ^{3}H-naloxone in rat brain homogenates. The concentration of drug producing 50% inhibition of specific naloxone binding was determined from log-probit plots utilizing at least five concentrations of each drug.

DRUG	Concentration Producing 50% Inhibition
Narcotics	
Morphine	6.5×10^{-9}
Methadone	1.5×10^{-7}
Codeine	1.0×10^{-5}
α-Blockers	
Phenoxybenzamine	9.2×10^{-7}
Phentolamine	5.6×10^{-6}
β-Blockers (10^{-5}M)	
Propranolol	No effect
Practolol	No effect
Other Agents (10^{-5}M)	
Br-LSD	No effect
Atropine	No effect
Promethazine	No effect

The specificity of the action of α-blockers on naloxone-binding in rat brain homogenates was established by the fact that β-adrenergic blockers and several other agents, which presumably block the effects of transmitters (other than the catecholamines) at the receptor level, failed to inhibit specific tissue binding of naloxone.

Discussion

The results of these studies indicate that α-adrenergic blockers, like the narcotics, reduce the binding of naloxone to rat brain. We know of no other non-narcotic compounds which have been shown previously to inhibit specific naloxone tissue binding. In view of these observations and our previous findings that α-blockers exert some antinociceptive activity, markedly potentiate morphine's effects, and attenuate the expression of the narcotic withdrawal syndrome (1,2,3), the possibility that α-adrenergic blockers and the narcotics are closely related should be considered. Much further work will be required to assess more

fully the degree of similarity between these classes of compounds.

Acknowledgments

This research was supported by USPHS grants DA-00109 and DA-00259.

T.J.C. is a recipient of Research Scientist Development Award MH-07180.

References

1. T. J. Cicero, Arch. Int. Pharmacodyn. Ther. In press.

2. T. J. Cicero, E. R. Meyer and B. R. Smithloff, J. Pharmacol. exp. Therap. 189, 72 (1974).

3. T. J. Cicero, E. R. Meyer and R. D. Bell, Neuropharmacology. In press.

4. C. B. Pert and S. H. Snyder, Science 179, 1011 (1973).

5. C. B. Pert and S. H. Snyder, Proc. Nat. Acad. Sci. 70, 2243 (1973).