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EFFECT OF APOMORPHINE ON OPIATE RECEPTORS IN THE RAT BRAIN

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Interest in the long familiar psychoparmacologic agent apomorphine has recently increased considerably. This is because of the recently discovered property of apomorphine of reducing withdrawal symptoms when the consumption of narcotics and alcohol is stopped and of reducing the dependence of chronic alcoholics and drug addicts on them [4]. A connection has also been found between the pharmacological action of apomorphine on animals in the laboratory, expressed as potentiation of some effects of morphine by apomorphine and inhibition of others [3, 6, 10].

Apomorphine has been shown to be an agonist of dopamine and to act on presynaptic dopamine receptors [3]. Morphine also is known to exert a marked influence on dopamine receptors. The connection observed between the effect of morphine and that of apomorphine is therefore nowadays explained by their interaction with these receptors [9]. Moreover, there is a tendency to explain most of the effects of apomorphine by its influence on the dopamine receptors of the brain [2]. However, there is some evidence which is not in harmony with the existing views [5, 14]. Analysis of these data, together with the well-known fact of the opiate activity of morphine, thus leads to the suggestion that apomorphine has a direct influence on the opiate system of the brain. Although in some investigations the question of the possible action of apomorphine on brain opiate receptors has been examined [13], no direct proof of this has so far been obtained.

The object of this investigation was accordingly to study interaction between apomorphine and the opiate receptors of the brain by direct methods.

EXPERIMENTAL METHOD

Male Wistar rats weighing 150-200 g were used.

To analyze the ability of apomorphine to bind with opiate receptors, methods of competitive replacement of the "pure" morphine antagonist naloxone [7,8(n)-^{3}H] or of the highly active D-analog of leucine-enkephalin — D-ala^2-[tyrosyl-3,5-^{3}H]-enkephalin (5-D-leucine) — from opiate receptors of the rat brain by apomorphine were used. The membrane fraction of the rat brain cells was obtained [11] as follows; a rat was decapitated, the brain was quickly removed in the cold, the cerebellum, which does not contain opiate receptors [7], was cut off, and the rest of the brain was homogenized in 45 volumes of cold Tris-HCl buffer, pH 7.7, in a homogenizer of Dounce type (glass—Teflon). The resulting suspension was centrifuged at 30,000g for 20 min at 4°C. The residue was suspended in the original volume of the same buffer and kept for 40 min at 37°C. The suspension was then recentrifuged under the same conditions. The residue thus obtained was resuspended in 50 mM Tris-HCl, pH 7.7 (25°C), containing 10^{-4} M EDTA, or in 50 mM Tris-HCl, pH 7.7 (25°C), containing

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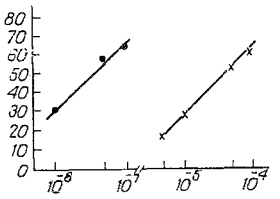

Fig. 1

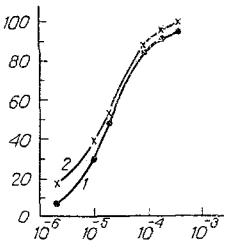

Fig. 2

Fig. 1. Comparative ability of naloxone and apomorphine to displace 5-D-leucine from opiate receptors of rat brain. Abscissa, concentration of naloxone and apomorphine in reaction mixture; ordinate, inhibition of specific binding of label with opiate receptors of rat brain (in percent of control). Reaction mixture also contains bacitracin (50 μg/ml) to prevent proteolytic degradation of the label. Each value is the mean of two determinations, differing from each other by less than 10%. Concentration of label in reaction mixture 1.5×10^{-9} M.

Fig. 2. Displacement of labeled naloxone by apomorphine from opiate receptors of rat brain. Abscissa, apomorphine concentration (in M); ordinate, inhibition of specific binding of labeled naloxone with opiate receptors of rat brain (in percent of control). 1) mixture did not contain Na^+, 2) contained 100 mM Na^+.

10^{-4} M EDTA and 100 mM NaCl. The reaction of binding of apomorphine with the opiate receptors was conducted in darkness (because of the photosensitivity of apomorphine) at 25°C for 40 min. The reaction mixture contained: 1.7 ml of membrane protein suspension, 1.6 nM labeled naloxone, or 3.1 nM of the labeled analog of Leu-enkephalin. Specific binding of the label with opiate receptors was determined as the difference in binding of the label in the presence and absence of 20 μM naloxone in the reaction mixture. The reaction was stopped by placing the samples on an ice bath. The samples were then quickly filtered (not more than 10 sec per sample) through glass filters of GF/C type (Whatman, USA). The filters were washed with 7.5 ml of cold (2°C) 50 mM Tris-HCl, pH 7.7, and transferred to flasks for measurement of their radioactivity. Into each flask 10 ml of standard toluene scintillator, containing 30% Triton X-100 and 0.05 M NH_4OH, was poured, the flasks were kept for 24 h at 20°C, after which radioactivity was measured on a type SL-3120 counter (France). By this method it was possible to obtain reproducible results with an efficiency of counting radioactivity of not less than 28%. Protein in the samples was determined by Lowry's method [8].

The following reagents were used: naloxone-[7,8(n)-^{3}H](16.4 Ci/mmole, from New England Nuclear: The analog of Leu-enkephalin (16 Ci/mmole), from the Radiochemical Centre, Amersham (England), both generously presented by G. Ya. Bokalkin; naloxone (Narcan) was from Winthrop Laboratories (USA); bacitracin 53,500 i.u./g) and Tris were from Sigma (USA); EDTA was from Serva (West Germany); 2,5-diphenyloxazole (PPO), p-bis-2,5-phenyloxazolylbenzene (POPOP), and Triton X-100 from Koch-Light Laboratories Ltd. (England).

EXPERIMENTAL RESULTS

The comparative ability of apomorphine and naloxone to displace competitively the analog of Leu-enkephalin from opiate receptors of the rat brain is shown in Fig. 1. Clearly the concentration of apomorphine displacing 50% of the label from receptors in an incubation medium not containing Na^+ was 42.0 μM. Hence, it follows that apomorphine is able to bind specifically with opiate receptors of the rat brain, but with much less affinity than the highly active morphine antagonist naloxone.

The ability of apomorphine to displace naloxone-[7.8(n)-^{3}H] competitively from the opiate receptors of the rat brain is shown in Fig. 2. Clearly the apomorphine concentration displacing 50% of labeled naloxone from receptors in medium not containing Na^+ (effective concentration, EC_{50}^{-NaCl}) was 20 μM. This concentration was much greater than the corresponding concentrations (EC_{50}^{-NaCl}) for morphine, naloxone, nalorphin, and Leu- and Met-enkephalin; α- and β-endorphins [11]. This is evidence of the comparatively low affinity of apomorphine for rat brain receptors which bind naloxone stereospecifically and reversibly.

The concentration of apomorphine displacing 50% of labeled naloxone from opiate receptors in an incubation mixture containing 100 mM NaCl (EC_{50}^{+NaCl}) was 17 μM. It follows from this that the sodium shift of effective concentrations, given by the ratio $EC_{50}^{+NaCl}/EC_{50}^{-NaCl}$, was 0.85. The value of this shift for the "pure" morphine antagonist, naloxone, is known to be 1.0, and for "mixed" antagonists it is 1.7-2.7; for antagonists and agonists it ranges from 3.3 to 6.0, and for "pure" agonists from 12 to 60 [12]. Consequently, by this criterion apomorphine more closely resembles the "pure" morphine antagonist. This is in good agreement with the observed depression by apomorphine of locomotor activity induced in rats by morphine [1], the development of the jump effect, characterizing the withdrawal state in animals [10]. These data also agree with the widely familiar clinical fact that apomorphine abolishes morphine and alcohol withdrawal symptoms in man [4].

On the basis of these results it can also be postulated that the effect of apomorphine on the dopamine system of the brain may be mediated through its effect on the opiate receptors of the brain.

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