EFFECT OF MORPHINE ON SENSITIVITY OF CORTICAL NEURONS TO ACETYLCHOLINE

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Most of the effects of morphine (analgesia, weakening of the summation ability of the CNS, potentiation of the action of narcotics, hypnotics, local anesthetics, etc.) [1, 3], are evidence of depression of the various functions of the CNS.

The principal hypothetical mediator of excitatory type in the CNS is acetylcholine (ACh) [6]. There is evidence that ACh mediates activating influences of the reticular formation and hypothalamus on the cerebral cortex [2, 9, 10].

It can be tentatively suggested that morphine affects the efficiency of cholinergic transmission by modifying the sensitivity of acetylcholine receptors in the nerve cell membrane. This hypothesis was tested by the present investigation. Chemicals were applied to single sensomotor cortical nerve cells of a rabbit by microiontophoresis.

EXPERIMENTAL METHOD

Experiments were carried out on 12 adult rabbits weighing 3-4 kg. Short-term hexobarbital anesthesia was used, only during the preparatory operations. The animals were immobilized with diplacin (5 mg/kg) and artificially ventilated. Fixation points of the skull in the stereotaxic apparatus were infiltrated with long-acting procaine.

Altogether 73 sensomotor cortical neurons were investigated. Action potentials (AP) of the neurons were derived extracellularly with one barrel of a 5-barrelled glass microelectrode, filled with 3 M NaCl. The other barrels of the microelectrode served for microiontophoresis and were filled with aqueous solutions of the following substances: morphine hydrochloride (0.05 M, pH 4.0), acetylcholine chloride (0.3 M, pH 4.0), and glutamic acid (0.5 M, pH 7.1). One of the barrels filled with 3 M NaCl was used to compensate current artefacts.

EXPERIMENTAL RESULTS

The predominant response of sensomotor cortical neurons to microiontophoretically applied morphine was a decrease in frequency of AP (43 of 73 neurons tested). Electrical activity of some neurons was increased (18 of 73) or unchanged (12 of 73) by application of morphine.

Besides sensitivity to morphine, sensitivity of all the neurons to ACh was studied. All possible combinations of responses of the neurons to morphine and ACh were observed (Table 1). Most frequently neuronal activity was inhibited by morphine and increased by application of ACh (24 of 73 neurons). Responses of many neurons to morphine and ACh were of the same modality. These were mainly a group of neurons whose electrical activity was reduced by the action of both agents (16 of 73 cells).

Antagonism was found between morphine and ACh when they were applied simultaneously to a neuron. ACh, applied by a microiontophoretic current of 60 nA, caused an increase in the frequency of AP typical of activation of muscarinic acetylcholine receptors (Fig. 1a). Morphine, applied by a weak current (8 nA), reduced the excitatory response of the neuron 80 sec after the beginning of ACh application. If the substances were applied to the cell in the opposite order, morphine first and ACh 76 sec later, the latter in general had no effect. The

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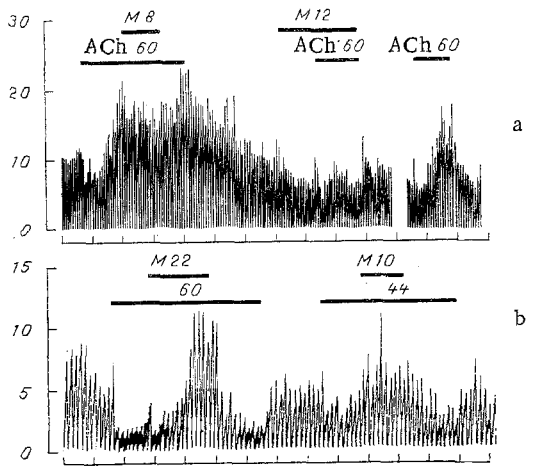

Fig. 1

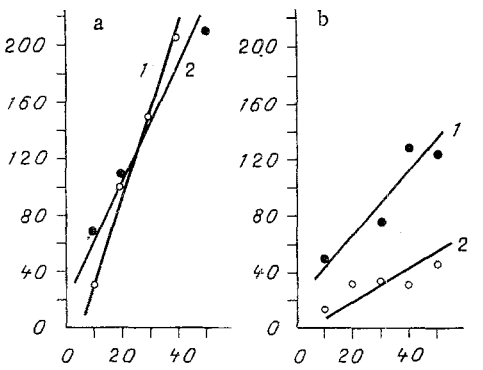

Fig. 2

Fig. 1. Depression of excitatory (a) and inhibitory (b) responses to ACh of a rabbit sensomotor cortical neuron by morphine. Abscissa, time (in min); ordinate, firing rate of neuron (spikes/sec). Horizontal lines — duration of microiontophoretic application of substances; numbers above them — strength of microiontophoretic currents (in nA). M) morphine. a) last application of ACh 5.5 min after end of preceding application of morhine. Remainder of explanation in text.

Fig. 2. Dose dependence of excitatory effect of glutamic acid (a) and ACh (b) before (1) and during (2) microiontophoretic application of morphine to a neuron. Abscissa, doses of drugs (in units of microiontophoretic current — nA); ordinate, increase in discharge frequency (in per cent of initial value, taken as 0). Each point on graph corresponds to peak value of effect of drug with corresponding current. Each dose of drug was applied for 60 sec. Morphine was applied by a current of 50 nA. Morphine applied alone did not change the spontaneous firing rate of the neuron.

excitatory response of the neuron to ACh was restored 5.5 min after the end of morphine application. A similar reduction of complete blocking of excitatory responses of neurons to ACh by morphine was observed in 7 of the 11 neurons tested.

We know that some neurons, located mainly in the surface layers of the cortex, respond to microiontophoretic application of ACh by a decrease in firing rate. Morphine reduced ACh-induced depression of neuronal activity in 9 of the 14 neurons tested. A typical example of depression of a neuronal response of inhibitory type to ACh by morphine is shown in Fig. 1b.

Thus morphine may reduce responses of neurons to ACh. Nevertheless, receptors of the neuronal membrane which are sensitive to morphine cannot evidently be completely identical with acetylcholine receptors. This is shown, first, by the fact that responses of the same neuron to morphine and ACh are not always identical in direction, and second, that the muscarinic cholinolytic atropine, if injected intravenously or applied microiontophoretically in a dose blocking responses of neurons to ACh, changed neither the excitatory nor the inhibitory responses to morphine (5 neurons). Sometimes morphine was observed to inhibit the excitatory response of the neurons to another hypothetical CNS mediator, namely glutamic acid. However, this effect arose only when morphine was applied in large doses (microiontophoretic current of over 50-100 nA), with a local anesthetic (membrane-stabilizing) action, expressed as a decrease in the amplitude and frequency of AP or even to complete cessation of the cell discharge.

By contrast with this, small doses of morphine (current up to 50 nA), without any local anesthetic action and not reducing the spontaneous firing rate of the neuron, were sufficient to cause marked inhibition of neuronal responses to ACh. It will be clear from Fig. 2a that the dose—effect curve of the excitatory response of the neuron to glutamic acid was unchanged by morphine, applied by a considerable current (50 nA). However, morphine applied by a current of the same magnitude was sufficient to reduce the response of the same neuron to ACh considerably. This was shown by a shift of the dose—effect curve in Fig. 2b to the right.

TABLE 1. Relations between Different Types of Responses of Sensomotor Cortical Neurons to Microiontophoretic Application of Morphine and ACh

ACh	Morphine			
	+	−	no effect	morphine
+	7	24	7	38
−	9	16	1	26
No effect	2	3	4	9
Total . . .	18	43	12	73

Legend. Numbers indicate numbers of neurons, +) increase in frequency of AP, −) decrease in frequency of AP.

The effect of depression of neuronal responses to ACh by morphine is thus due, not by direct inhibition of the mechanism of AP generation or reduction of Na^+-permeability, as some workers have postulated [8], but to the modulating effect of the drug on acetylcholine receptor activity. Definite interaction evidently takes place between the opiate receptor and the acetylcholine receptor. Correlation has been found between the ACh concentration and the number of opiate receptors in different parts of the brain [7].

Many studies have shown that cholinolytics not only do not possess analgesic properties, but indeed they can cause hyperalgesia and prevent the analgesic effect of cholinomimetics [4, 5]. The central cholinolytic action of morphine most probably makes a contribution to inhibition and dysfunction of higher forms of nervous activity which accompany the opiate intake.

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