A NEW PRINCIPLE OF EVALUATION OF MORPHINE AS AN ANALGESIC

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The range of experimental methods and models has expanded significantly in recent years, so that not only can the analgesic effect of neurotropic drugs be assessed in the traditional quantitative manner, but its dependence on the subject's initial state can also be determined [4, 7] and systemic, receptor, and other complex processes lying at the basis of drug-induced analgesia can be revealed [3, 5, 6]. Until now, however, it has remained difficult to analyze the effect of drugs on different stages of pain integration in the CNS, preceding the external manifestations of the nociceptive response and determining their character.

In recent investigations devoted to clinical aspects of the psychophysiology of pain and analgesia, a new approach based on Clark's theory [8] has begun to be used. This theory defines the role of interaction between the stages of "measurement" and "evaluation" of nociceptive stimuli in the formation of an individual's ability to respond to pain, using verbal criteria [10-12]. This approach in its original version has not yet been applied in experimental investigations, which have the undoubted advantage of the ability to reproduce a nociceptive response over a very wide range and to evaluate it in terms of objective features of the response to pain.

In this investigation the possibility of utilizing the principles of Clark's theory in experimental investigations was studied, i.e., the possibility of extrapolating them to the analysis of the neuropsychophysiological mechanisms of the analgesic action of morphine, as a standard analgesic, on a standard experimental model.

EXPERIMENTAL METHOD

Stimulation of the base of the tail, with gradually increasing strength, was applied through bipolar subcutaneous electrodes to 60 conscious rats. The intensity of stimuli not inducing changes in the animals' behavior (0 points) and also giving rise to the development of three types of responses, characterizing different levels of pain integration in the CNS, was determined [2, 9]: the tail shock reflex, a combination of generalized motor manifestations, and vocalization, assessed at 1, 2, and 3 points respectively. Each response was reproduced 5 times with an interval of 2 min in rats of the control group and 3 times in the animals before and after injection of morphine hydrochloride (5 mg/kg). The total number of tests in the control and experimental groups was 1500 and 500 respectively. The data were analyzed by Student's test and by the use of quantiles of the normal distribution [1].

EXPERIMENTAL RESULTS

Morphine significantly ($p < 0.001$) raised the thresholds of stimuli inducing a vocal response (from 0.5 ± 0.04 to 0.75 ± 0.07 mA) and, to a lesser degree, the tail shock reflex (from 0.20 ± 0.03 to 0.10 ± 0.02 mA). The results of this traditional evaluation, based on average tendencies, are known to give some idea of the quantitative characteristics of the effects of a drug, but they shed no light on the psychophysiological basis of its realization. Meanwhile even the simple comparison of these results with those of analysis by another, less trivial method and, in particular, with the aid of a matrix of states revealing the mode of the stochastic distribution of nociceptive responses of varied intensity (in %) depending on

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TABLE 1. Evaluation of Analgesic Effect of Morphine by Means of a Matrix of States

Experimental conditions	Intensity of nociceptive response, points	Intensity of stimulation, μA				
		0,2	0,3	0,4	0,6	0,8
Control	0	39	7	1	0	0
	1	46	45	18	5	2
	2	4	22	32	22	9
	3	11	26	49	73	89
Morphine (5 mg/kg)	0	55	34	6	0	0
	1	45	49	49	23	0
	2	0	17	45	57	64
	3	0	0	0	20	36

Legend. Data given comprise number of cases (in %) in which nociceptive responses of varied intensity were observed to stimulation of the base of the rat's tail.

the intensity of stimulation, clearly illustrates the complex character of the effect of morphine on the stages of pain integration preceding its external manifestations. The matrix of states shows (Table 1) that in the majority of animals, during stimulation with an intensity of, for example, 0.2 mA, the threshold level for tail shock reflex in accordance with average tendencies, it was indeed this response which was recorded. In that case the absence of a response to stimulation with an intensity of 0.2 mA (39%) or, conversely, the development of generalized motor manifestations and of vocalization (15%) can be interpreted as atypical (mistaken). During exposure to nociceptive stimulation the number of these "mistakes" may reach 50%, and may differ significantly in response to stimulation of different intensities (Table 1). The cause of the "mistakes" is evidently the variability of response both of different animals and of the same animal during repeated testing. In turn, the effect of morphine in relation to nociceptive responses, which were assessed at 1, 2, or 3 points, was clearly inconstant. However, it is only by the use of Clark's theory that the action of morphine on the processes of measurement and evaluation of pain stimuli can be analyzed and the nature of variability in response to pain revealed.

Extrapolation of the principles of Clark's model to the results of the present experiments is expressed graphically in Fig. 1, I. Measurement of the applied stimulus included its comparison with a certain internal scale of the body (horizontal line) and was accompanied by an error described by the curve of the normal distribution (the bell-shaped curves a and b). It will be evident that typical results of measurements of each successively applied stimulus of increasing intensity lie closest to the peaks of curves a and b.

The character of the external response to each stimulus is determined by its assessment, i.e., by comparison of the results of measurements of the magnitude of the stimulus with the limits present on the internal scale, and characterizing the transmission from one type of response, designated in points, to another type (vertical lines 0-1 and 2-3). For example, if during measurement a signal with intensity of 0.8 mA on the internal scale is placed in the region lying on the right of the 2-3 boundary, external manifestations will correspond to a typical emotional-painful response with vocal reaction. However, the same stimulus in some cases may be classed on the internal scale in the region lying on the left of the 2-3 boundary, and under these circumstances there will be no vocalization.

Our data, presented by Clark's method, show that the greater the intensity of stimulation, the more accurately the animals assessed the stimuli applied, and the more adequately they responded to them. For instance, during stimulation of the base of the tail with an intensity of 0.8 mA almost 90% of rats developed the vocalization characteristic of that intensity (Table 1), because the peak of the curve b, corresponding to measurements of a stimulus with an intensity of 0.8 mA, lies at a distance of 1.2 conventional units of the internal scale to the right of the 2-3 boundary (Fig. 1, I). In response to stimulation with an intensity of 0.2 mA the number of "mistakes" in the animals' response was considerably greater — in 39% of cases a nociceptive tail shock reflex at the threshold level was absent. The

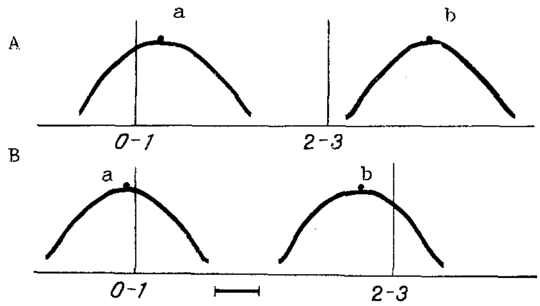

Fig. 1. Analysis of analgesic action of morphine using the principles of Clark's theory [8]. A) Control, B) morphine (5 mg/kg). Horizontal lines — internal scales for measuring nociceptive stimuli (calibration — 5 conventional units); curves a and b) "mistakes" of measurement of stimuli with intensities of 0.2 and 0.8 mA respectively; vertical lines indicate boundaries of transitions from one type of response to another (in points).

reason was that the peak of the curve a, corresponding to measurement of a stimulus with an intensity of 0.2 mA, lies on the right, much closer to the 0-1 boundary, at a distance of only 0.3 conventional unit. The distance between the peaks of curves a and b is 3.2 conventional unit. Within this range lie curves which are not shown in order to simplify the graph in Fig. 1, I, and which demonstrate the results of measurement of stimuli with intensities of 0.2 to 0.8 mA. The length of the interval between the peaks of curves a and b reflects the ability of the organism, to use Clark's terminology, to distinguish between stimuli of different intensity. Finally, the distance along the internal scale between the boundaries of transitions from response of type 0 to type 1 and from type 2 to type 3 (2.3 conventional units) characterizes the ability of the organism to generate responses to stimuli measured in different ways, appropriate to the results of measurement.

Morphine (Fig. 1, II) caused a shift of the peak of curve A, corresponding to a stimulus with intensity of 0.2 mA, to the left of the 0-1 boundary by 0.1 conventional unit. The peak of curve b also was shifted to the left of the 2-3 boundary by 0.4 conventional unit. These shifts, identical in direction but differing in degree, determined the greater decrease in the frequency of vocalization in response to a stimulus of 0.8 mA than of the tail shock reflex to a stimulus of 0.2 mA (Table 1). On the whole, the ability of the animal to distinguish between stimuli of different intensity was reduced by morphine on account of a decrease in the distance between the peaks of curves a and b to 2.7 conventional units. Meanwhile the distance between the 0-1 and 2-3 boundaries increased to 3.1 conventional units, evidence that ability to assess the measured stimulus was disturbed by the action of the drug.

The results thus showed that the principles of Clark's theory can be extrapolated for experimental investigations and that it is informative for the analysis of the neuropsychophysiological mechanisms of opiate analgesia. The analgesic action of morphine was found to be due both to impairment of ability to distinguish nociceptive stimuli and to a disturbance of formation of a response that is adequate with respect to the results of measurement of the stimulus inducing it. It must be emphasized that morphine changes both these basic stages of pain integration, preceding its external manifestations, in the same direction, with the result that a marked analgesic effect of the drug is formed. From these standpoints the established ideas regarding the preferential direction of the action of morphine on highly integrated components of pain [2] can be explained by inhibition of measurement of, in particular, strong nociceptive stimuli. Meanwhile the impairment of ability to distinguish stimuli of different intensity leads to an increase in the number of cases of atypical (inadequate) response to nociceptive stimulation of threshold strength, under the influence of morphine. In our view, the use of the principles of Clark's model makes it possible to analyze the results of experimental investigations of the mechanisms of action of analgesics, and it may be an important tool with which to discover the psychophysiological features of the analgesic effect of opioids and other classes of drugs.

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OPEN FIELD BEHAVIOR OF RATS AFTER BILATERAL COAGULATION OF THE LOCUS COERULEUS

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It was shown previously that emotional stress in animals causes increased permeability of the blood–brain barrier, and injury to the intracerebral vessels in the mesencephalic reticular formation, with resulting death of neurons. It has been postulated that it is destruction of the mesencephalic reticular formation that is largely responsible for behavioral changes caused by emotional stress [1, 2, 5]. Since information has been obtained that the locus coeruleus participates in the regulation of functions of the blood–brain barrier [2, 8-9], it might be supposed that the injuries to blood vessels observed in the mesencephalic reticular formation are also the result of dysfunction of the locus coeruleus induced by emotional stress.

The aim of this investigation was to test this hypothesis by assessing behavioral changes developing after bilateral injury to the locus coeruleus, using the open field test. Unlike workers who studied this problem previously [3, 4], we modernized the method used for the open field test to some extent by introducing into it a sudden stress stimulus in order to simulate the "start reflex," known in neurology as a protective response linked with the function of the tegmentum mesencephali.

EXPERIMENTAL METHOD

Experiments were carried out on 33 male Wistar rats weighing 150-320 g. The animals were tested once in the course of 5 min by the modified open field test. The arena, measuring 60 × 60 cm, was divided into 16 squares, each side of which measured 20 cm. Above the center of the arena hung a 40-W lamp at a height of 80 cm. The test was carried out in a dark, soundproofed room.

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