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MULTIFACTORIAL ANALYSIS OF THE EFFECT OF FENTANYL ON NOCICEPTIVE HEMODYNAMIC RESPONSES

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Abolition of responses of the cardiovascular system to nociceptive stimuli is one indicator of adequate pharmacologic analgesia [1, 5, 7]. However, existing data on the ability of specific pain-relieving drugs (narcotic analgesics) to stabilize the hemodynamics during nociceptive stimulation are highly contradictory [6, 8, 9], due to differences in the conditions of the experimental and clincial investigations and the absence of analysis of the action of the drugs depending on the initial state of the recipient. In turn, objective analysis of the influence of the original state on the effect of the drug calls for a study of relations between background values of the whole range of parameters recorded and their changes after administration of the drug [4]. It is virtually impossible to discover complex relationships of this kind by traditional analysis of data based on average tendencies.

In the investigations described below the effect of fentanyl on nociceptive hemodynamic responses was studied depending on the initial state of the animals, and a comparative evaluation of the data was made by determination of average tendencies and by the multiple step-by-step bilinear regression methods.

EXPERIMENTAL METHOD

The pulp of the upper canine teeth was stimulated in 14 experiments on eight cats thorough chronically implanted electrodes. The emotional reactivity of the animals was assessed virtually in accordance with special scales [3], the arterial blood pressure (BP) and intersystolic intervals (ISI) were recorded, and the values of the cardioinhibitory baroreflex [2] were calculated. Fentanyl (solution in ampules) was injected intravenously in doses of 1 to 30 μg/kg body weight.

Multifactorial analysis of the data was carried out on the SM-3 computer, using a multiple step-by-step regression program. Regression equations were constructed [4] to estimate dependence of changes in hemodynamic responses not only on dose and time after injection of fentanyl, but also on the initial state of the animals. Parameters (14) of emotional reactivity, reflex mechanisms of regulation of the hemodynamics — intrinsic (baroreflex) and coupled (responses of BP and ISI to stimulation of the dental pulp) were analyzed as factors reflecting intial state (Table 1). At the first and each subsequent step of its program, the factor correlating most strongly with the effect of the drug was introduced into the equation from the number of factors not taken into account by the program previously. Ultimately factors with a level of significance not below 0.01 were included in the final form of the equations.

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TABLE 1. Effect of Fentanyl on Background Hemodynamic Parameters and Nociceptive Responses to Stimulation of Dental Pulp in Cats

Experimental conditions	BP, mm Hg	ISI, msec	Cardio-inhibitory baroreflex, msec/mm Hg	Hemodynamic responses		Emotional-behavior response (mean intensity of stimulation, in thresholds, required to produce the given feature)								
				Elevation of BP, mm Hg	Shortening of ISI, msec	level I			level II			level III		
						mouth opening reflex	licking	piloerection	changes in respiration	unoriented movements	defensive movements	scratching	running	vocalization
Control	121±7	495±85	8,4±1,3	27±6 (14—54)	205±33 (120—245)	1,0	1,2	2,2	2,1	2,2	5,9	6,8	7,9	7,6
Fentanyl in dose of 15 mg/kg:														
10 min	132±15	510±97	6,0±1,2	18±7	270±44	1,0	1,3	2,5	2,6	2,3	6,6	7,9*	8,4	10,1
25 min	129±16	510±96	3,7±0,9*	10±3* (9—16)	220±28 (140—310)	1,0	1,4	2,6	2,8*	2,9*	7,2*	10,2*	8,9*	—
Fentanyl in dose of 30 mg/kg:														
10 min	152±9*	380±41	3,6±1,8*	11±3* (6—18)	145±15 (90—180)	1,2	1,6	3,1*	3,1*	2,6	7,4*	11,8*	12,2*	—
25 min	142±9*	445±81	3,4±0,8*	8±3* (0—16)	140±9 (120—165)	1,3	1.6	3,4*	3,3*	3,5*	7,5*	12,4*	12,6*	—

Legend. Asterisk indicates significant changes compared with control ($P < 0.05$), a dash indicates that the feature was not produced. Range of values of parameters shown in parentheses.

EXPERIMENTAL RESULTS

Traditional evaluation vased on average tendencies revealed no significant effect of fentanyl, in the doses tested, on nociceptive tachycardial responses. Meanwhile considerable variability of responses of ISI was found in different animals (Table 1), evidence of individual differences in the effect of fentanyl.

By the use of the multiple step by step regression method to analyze the data not only could the main trend in the action of fentanyl in all the animals be established, but individual differences in the effect of the drug on changes in ISI due to stimulation of the dental pulp could also be demonstrated. The results of multifactorial analysis are given in Fig. 1. The origin of all the lines on the graphs reflects the initial range of real values of responses of ISI recorded in the experiments. Besides dose and time, parameters most significantly ($P < 0.001$) influencing the effect of fentanyl were selected by the program: the magnitude of the original tachycardial response and the intensity of emotional-behavioral manifestations in response to stimulation of the dental pulp. It will be clear from Fig. 1 that the action of fentanyl in a dose of 15 μg/kg, manifested as enhancement of the tachycardial responses, was most clearly exhibited against the background of a generalized (level III) behavioral resonse (Fig. 1a, 2). With an increase in the dose and time after injection of fentanyl this effect weakened and was replaced by inhibition of the responses of ISI that was exhibited most clearly during minor behavioral responses (Fig. 1b, 1).

The use of multifactorial analysis of the data thus revealed resistance, common to all animals, of the nociceptive tachycardial changes to the depressant action of fentanyl. At the same time it was shown that in highly emotional cats fentanyl, during the first few minutes after injection in a dose of 15 μg/kg, increased the responses of ISI. This complex dependence of the effect of the drug not only on dose and time, but also on the parameters of the initial state of the recipient, could not be detected when the effect was assessed by the usual methods.

Analysis of changes in nociceptive shifts of BP based on average tendencies indicated, at first glance, that fentanyl in doses of 15 and 30 μg/kg had a distinctly depressant effect (Table 1). Just as with the response of ISI, in this case also considerable individual differences were observed in the changes in the pressor shift. The use of multifactorial analysis was able to demonstrate that the inhibitory action of fentanyl on the response of BP was most marked in animals with high initial activity of their baroreceptor reflexes (Fig. 2b). Meanwhile, if values of the baroreflexes and pressor shifts were minimal in the control, fentanyl in a dose of 15 μg/kg caused virtually no change in nociceptive responses of BP (Fig. 2a).

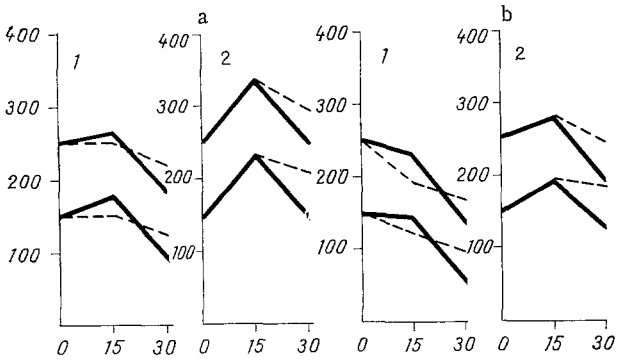

Fig. 1

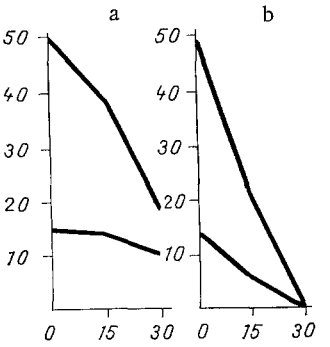

Fig. 2

Fig. 1. Effect of fentanyl on responses of ISI to stimulation of dental pulp in cats. Abscissa, dose of fentanyl (in μg/kg); ordinate, responses of ISI (in msec); a, b) 10 and 25 min respectively after injection of fentanyl; 1, 2) changes in parameter in animals under conditions of minimal and maximal emotional reactivity respectively.

Fig. 2. Effect of fentanyl on responses of BP to stimulation of dental pulp in cats. Ordinate, responses of BP (in mm Hg). 1, 2) Changes in parameter corresponding to minimal (5 msec/mm Hg) and maximal (15 msec/mm Hg) values of cardioinhibitory baroreflex in control. Remainder of legend the same as to Fig. 1.

Consequently, whereas the character of the effect of the analgesic on ISI shifts depends mainly on the animals' emotional reactivity, changes in responses of BP are determined by the initial values both of the responses themselves, and of the baroreflex, i.e., by other parameters of the animal's initial state.

Table 1 shows that changes in hemodynamic responses after injection of fentanyl took place simultaneously with inhibition of behavioral manifestations. To investigate correlation between the pain-relieving (emotiotropic) effect and the effect of the drug on nociceptive shifts of BP and ISI the following method was used: besides the 14 parameters of the animals' initial state already mentioned, changes in emotional-behavioral features evoked by fentanyl also were included in the regression equations as additional factors. Next, by means of the equations, the hemodynamic effects that would be produced on the assumption that the drug does not alter behavioral responses during stimulation of the dental pulp, were calculated. It was found that if no emotiotropic action of fentanyl was present, the effect on responses of BP and ISI would not differ from those observed experimentally. It could accordingly be suggested that the behavioral and the cardiovascular actions of fentanyl under conditions of nociceptive stimulation are separate entities.

Analysis of the action of fentanyl by multiple step-by-step regression thus showed that inhibition of the emotional-behavioral manifestations of the response to pain may be combined with preservation or even enhancement of nociceptive hemodynamic shifts. By multifactorial analysis the basic correlations could be detected among a large number of background parameters and their changes, and it showed that the use of this method to assess standard analgesics and new compounds can yield more detailed information on their effect on the various manifestations of the nociceptive response.

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INTERACTION BETWEEN KYNURENIN AND DIAZEPAM

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Diazepam, a universal anticonvulsant, prevents seizures induced by injection of L-kynurenin sulfate, an endogenous metabolite of tryptophan, into the cerebral ventricles of mice only in a dose as high as 30 mg/kg (ED_{50}), whereas against metrazol, maximal electric shock, and strychnine, its ED_{50}, according to our data and those published in the literature, is 0.5-1.0, 3.0-4.0, and 9.0-10.0 mg/kg respectively. It has been suggested that the uniquely low activity of diazepam against kynurenin may perhaps be connected with the fact that the latter, with a similar structure to that of diazepam, either as a ligand reduces binding of diazepam with benzodiazepine receptors or, as a modulator with no effect on binding, depresses the physiological effect of this binding.

It was accordingly decided to study interaction between kynurenin and diazepam in tests other than those with kynurenin-induced seizures. This paper describes an investigation into this problem.

EXPERIMENTAL METHOD

Experiments were carried out on male SHR albino mice and C57BL/6 black mice weighing 16-18 g, from the "Rappolovo" nursery, Academy of Medical Sciences of the USSR, in September-December. An aqueous solution of L-kynurenin sulfate (subsequently described as kynurenin), from Sigma (USA), was injected into the cerebral ventricles of waking animals by means of a semiautomatic apparatus. Control mice received injections of 5 µl of physiological saline. The method was described in detail previously [13]. Diazepam (packed in ampuls, from Gedeon Richter, Hungary), metrazol, and caffeine (caffeine sodium-benzoate or base) in distilled water were injected intraperitoneally in a volume of 1% of body weight. Diazepam was injected 30 min before metrazol or caffeine, and 20 min before determination of orienting motor activity; kynurenin was injected 1 min before caffeine or metrazol and 10 min before determination of orienting motor activity. The convulsant effect of metrazol and caffeine was assessed on the basis of five criteria: the latent period of clonicotonic seizures, the number of animals with clonic seizures in the group, the number of animals with tonic extension in the group, mortality, length of survival. Equally effective doses (ED_{90}, intraperitoneally) were used: the metrazol 80 mg/kg, of caffeine sodium-benzoate 170 mg/kg, and of caffeine base 250 mg/kg. Orienting motor activity was recorded in a single mouse for 2 min in a metal chamber measuring 20 × 15 × 10 cm, at the number of times the mouse crossed lines dividing the floor into four rectangles (locomotion), and as the number of times the mouse stood up on its hind limbs. A conflicting situation test [8, 9], which has proved to be adequate and reliable for the study of benzodiazepine and other tranquilizers, was carried out in a chamber consisting of light (27 × 28 × 27 cm) and dark (27 × 16.5 × 27 cm) parts. Locomotion and standing movement of a single mouse were recorded for 2 min in both compartments, and the number of crossings from one compartment into the other through a gate 10 cm wide and 6 cm high was recorded. The mice were placed in a corner of the light compartment. Diazepam and kynurenin were injected simultaneously 30 min before the test.

The alternative data were compared by means of tables [1] and the graduated data by Student's t test. Each group of mice contained 10-12 animals.

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