EFFECT OF DIPYRIDAMOLE ON BLOOD LEVELS OF OPIOID PEPTIDES AND ALPHA-INTERFERON

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A noteworthy feature of the spectrum of pharmacological activity of the coronary vasodilator dipyridamole, widely used in medicine, is its recently discovered antiviral properties [11]. These effects are based on induction of interferon, 85% of it being of the "alpha" subtype [10]. On the other hand, besides its antiviral, cytostatic, and anticellular action, alpha-interferon itself (α-IFN) also exhibits neurotypic activity and, in particular, it possesses opiate-like properties [7]. The effect of α-IFN on opioid systems is probably realized through interaction with specific, opioid-binding sites on the membranes [4, 7]; the character of its binding by opioid receptors depends on the nature of the immune polypeptide and on the receptor selectivity of the ligand used for testing [1, 5]. Investigation of interaction of α-IFN and the ligand component of opioid systems consists essentially of determination of the effect of opioids on the production of this glycoprotein as a hypothetical mechanism of the immunosuppressive action of morphine agonists [8]. The opposite relationships have received much less study: the liability of endogenous opioids to changes in their levels in response to the entry of α-IFN into the body [6].

Dipyridamole may perhaps exert its influence on opioid systems by another mechanism. It has been shown that one of the main points of application for the action of this coronary vasodilator is the *c*AMP system [2], for which, in turn, an influence on opioid systems has been described [12].

Because of the inadequate scientific data on this subject, it was decided to study the character of the effect of dipyridamole on opioid peptides from the standpoint of interaction between the nervous and immune systems.

EXPERIMENTAL METHOD

Experiments were carried out on volunteers of both sexes (two men and seven women) aged from 30 to 65 years. The subjects had a history of repeated diseases of a viral nature. During the two weeks before the experiment, the subjects were in normal health. The initial data were obtained at 9 a.m., in a state of relative rest. Repeated determinations were made under similar conditions 24 h after taking dipyridamole (Curantyl, from "Arzneimittelwerke," East Germany), according to the scheme suggested in [10]: 100 mg in the course of 2 h.

To study the ability of lymphocytes to produce α-IFN, cells isolated from venous blood in a Ficoll–Verografin gradient (10^6 cells/ml) were incubated for 24 h at 37°C in medium 199 with 10% homologous serum in the presence of Newcastle disease virus (multiplicity of infection 10 PFU per lymphocyte). The cells were sedimented by centrifugation at 600*g* for 40 min. The supernatant was kept until testing at 4°C for not more than 2 weeks. The α-IFN level in the supernatant, and also the serum IFN level, were estimated on the basis of inhibition of the cytopathic action of vesicular stomatitis virus in a culture of human embryonic M-19 diploid cells [9]. The unit of activity of IFN was taken to be the reciprocal of the dilution giving 50% protection of cells of the monolayer against the action of the test virus.

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TABLE 1. Effect of Dipyridamole on α-IFN Production by Lymphocytes and on Plasma Opioid Peptide Concentrations ($M \pm m$, $n = 9$)

Parameter	Group of subjects	
	control	Dipyridamole
Beta-endorphin, fmoles/ml plasma	4,3±0,4	3,8±0,4
Met-enkephalin, pg/ml plasma	79,5±7,6	110,3±4,8**
Serum IFN, units/ml	2,1±1,1	6,4±1,4*
α-IFN production, units/ml	7,9±1,3	12,6±1,2*

Legend. $*p < 0.05$, $**p < 0.01$ compared with control.

Concentrations of beta-endorphin and met-enkephalin in samples of blood plasma were determined with the aid of commercial radioimmunoassay kits ("Immuno Nuclear Corp.," USA). Radioactivity was counted on a "Minigamma" gamma-counter (LKB, Sweden), using a "Data Box" processor (Sweden) and calibration curve, in the form of a spline function, to determine concentrations. The results were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

Administration of dipyridamole led to an increase in the met-enkephalin but not the beta-endorphin level in the blood plasma (Table 1). The changes observed theoretically reflect increased release of met-enkephalin from the cells synthesizing it. It is not yet possible to characterize unequivocally the origin of the met-enkephalin in blood plasma. Differences in the effect of dipyridamole on these peptides are probably due to the fact that they belong to difference classes of endogenous opioids: whereas beta-endorphin is synthesized from a high-molecular-weight precursor, namely pro-opiomelanocortin, met-enkephalin belongs to the proenkephalin family [13].

Opioid systems are known to function through the realization of the self-regulation principle [3, 14], and the results of this investigation accordingly suggest that dipyridamole led to blockade of opioid receptors. Consequently, its action on opioid receptors was antagonistic in character. On the basis of data in the literature a more accurate suggestion can be put forward regarding the activated type of opioid binding sites: a comparative regional distribution of peptides of the proenkephalin and pro-opiomelanocortin families, on the one hand, and of receptor subpopulations on the other hand; other data also are evidence of the preferential structural-functional association of met-enkephalin with delta-receptors and of beta-endorphin with the mu-system [15].

The data described above are not only theoretically important for an explanation of the change in the met-endephalin level through an influence on delta-receptors, but they also suggest the mechanisms of the effect of dipyridamole on the opioid pentapeptide. Although the possibility of direct interaction of dipyridamole with delta receptors cannot be completely ruled out, while recalling, however, that its chemical structure differs considerably from that of the opiates, it seems preferable to admit the intervention of a certain intermediate stage. As was stated above, both *c*AMP and α-IFN have a claim for this role [2, 7, 10, 12]. The results of pharmacological experiments [12] show that the effects of *c*AMP are largely mediated through mu-receptors which, according to the arguments presented above, are not involved in the action of dipyridamole on the met-enkephalin level. Consequently, the most likely mechanism of the effect of the coronary vasodilator is through activation of the endogenous α-IFN system.

A raised serum IFN level, together with increased ability of the lymphocytes to produce α-IFN, was found in samples of the blood used to determine opioid peptide concentrations (Table 1). These results agree in principle with data in the literature [10], but stimulation of IFN by dipyridamole was weaker. A possible cause of quantitative differences is the existence of signs of secondary immunodeficiency in the group of subjects, as indicated by their medical history (see above). The trend of changes in the α-IFN level in response to dipyridamole, in agreement with data in the literature, also is evidence that the quantitative differences cannot have any fundamental importance for the nature of the problem of interconnection between opioids and α-IFN now being studied.

Correlation analysis of individual determinations of the met-enkephalin level and the ability of lymphocytes to produce α-IFN, by Spearman's method, revealed significant statistical correlation between these parameters, with a coefficient of correlation of $\rho = 0.69$ ($p < 0.05$).

The results thus indicate that endogenous α-IFN may exert an influence on opioid peptides, and they suggest that this immune glycopolypeptide performs the role of regulator of the functional state of enkephalin, but not endorphin, systems in the body.

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