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SUGGESTIVE EVIDENCE FOR RECEPTORS FOR MORPHINE AND METHIONINE-ENKEPHALIN ON NORMAL HUMAN BLOOD T LYMPHOCYTES[1]

JOSEPH WYBRAN,[2] THIERRY APPELBOOM, JEAN-PIERRE FAMAEY AND ANDRÉ GOVAERTS

From the Departments of Immunology and Rheumatology, Saint-Pierre Hospital, Université Libre de Bruxelles, 1000 Brussels, Belgium

This study reports the *in vitro* influence of morphine, dextromoramide, levomoramide, and methionine-enkephalin upon normal human T blood lymphocytes by using the active and total rosette tests. Morphine and dextromoramide inhibited the percentage of active T rosettes. This effect was completely reversed in the presence of naloxone, their specific antagonist. The specificity was further demonstrated by the absence of the effect of levomoramide, the inactive enantiomere, upon the rosette system. Methionine-enkephalin increased the percentage of active T rosettes. This effect was specifically inhibited by naloxone. These observations suggest that normal human blood T lymphocytes bear surface receptor-like structures for morphine, dextromoramide, and methionine-enkephalin. Such findings may provide a link between the central nervous system and the immune system.

Human T lymphocytes possess various surface markers. Among them, one is an antigen shared by both the brain and T cells (1). This relationship has also been clearly identified in the mouse with the θ-antigen. Human T lymphocytes are easily recognized by their specific ability to form rosettes with sheep red blood cells (SRBC) (2). Morphine, its analogs, and enkephalins have been mainly studied for their binding to neural cells and in other various isolated smooth muscle preparations (3, 4). In view of the relationship between T lymphocytes and the brain, the action of these drugs was investigated upon the T cell rosette system. Two assays were studied: the total T rosettes, which identify all T cells and the active T rosette test, which is a subpopulation of T cells with receptors of high affinity for SRBC (5, 6). This latter test is always done under suboptimal conditions like low ratio of SRBC per lymphocytes and short incubation periods between SRBC and lymphocytes. The present observations suggest that normal human T lymphocytes bear surface receptors for morphine and methionine-enkephalin.

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[1] This work was supported by a grant of the Fonds de la Recherche Scientifique Médicale.
[2] Address for correspondence: Doctor Joseph Wybran, Service d'Immunologie et de Transfusion Sanguine, Hôpital Saint-Pierre, 322 Rue Haute, 1000 Bruxelles, Belgium.

MATERIALS AND METHODS

Peripheral blood mononuclear cells of normal subjects were isolated after centrifugation over a Ficoll-Hypaque gradient. After washings, the cells were adjusted to a final concentration of 15×10^6 cells/ml in medium RPMI 1640 with *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) (Gibco Biocult). Five hundred thousand cells were used for each test (always performed in duplicates); they were incubated with 0.05 ml of the drug at 37°C. The drugs were dissolved in medium RPMI 1640 with HEPES. The following drugs were used in this study: morphine chlorhydrade (Bios Lab., Brussels) at final concentration between 10^{-12} M and 10^{-7} M, naloxone chlorhydrate (Endolab.) at concentrations between 10^{-7} and 10^{-6} M, methionine-enkephalin (Peninsula Lab., San Carlos, Calif.) at concentrations between 10^{-10} and 10^{-4} M, dextromoramide and levomoramide (kindly provided by Janssen Pharmaceutica, Beerse, Belgium) at concentrations between 10^{-12} and 10^{-7} M. After 1 hr of incubation the cells were washed and assayed for rosette formation by using the active T test, the total T test, and, in preliminary experiments, the EAC rosette test (erythrocyte-antibody-complement).

All the rosettes tests were done according to previously published methods (7). Briefly, for detecting the active T rosettes, the lymphocytes were incubated for 1 hr in an equal volume of 0.033 ml of heat-inactivated absorbed fetal calf serum (Gibco-Biocult); then, SRBC in saline were added to obtain a final ratio of eight SRBC to one lymphocyte. The tubes were centrifuged at 200 × G for 5 min and gently resuspended. An aliquot of cells was taken, put in a hemocytometer, and active rosettes were counted with a light microscope. For the total T rosettes, 0.033 ml of fetal calf serum was added to the lymphocytes as well as 0.033 ml of SRBC (final ratio: 100 SRBC for 1 lymphocyte). The tubes were centrifuged for 5 min at 400 × G and left vertically overnight in a rack at 4°C. Then, after gentle resuspension, an aliquot of cells was taken for counting the total T cell rosettes.

In some experiments, shorter incubation times (5 min, 15 min, 30 min) with similar concentrations of morphine or methionine-enkephalin were performed, followed by washings and determination of the percentage of active T rosettes. In other experiments, the cells, first incubated for 1 hr with one drug (morphine 10^{-7} M or dextromoramide 10^{-7} M or methionin-enkephalin 10^{-7} M) were further incubated for another hour at 37°C in the presence of its antagonist (naloxone 10^{-6} to 10^{-12} M). After washings, the cells were assayed for active and total T rosette tests. Experiments with simultaneous incubation for 1 hr of the drugs and their antagonist were also performed. The statistical analysis of the data was done with the Student *t*-test for paired data.

TABLE I

Effect of various drugs on the percentages of active and total T rosettes (±S.D.)[a]

	Drug Concentration (M)									
	0	10^{-12}	10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}
I. Active T rosettes										
Morphine	23.7 ± 3.0	23.2 ± 3.3	22.2 ± 4.0	16.5 ± 1.3**	16.1 ± 3.5**	15.0 ± 4.1**	15.5 ± 2.6**	N.D.[b]	N.D.	N.D.
Dextromoramide	26.8 ± 1.8	27.8 ± 1.8	26.8 ± 3.2	22.8 ± 2.1	23.0 ± 2.3***	22.2 ± 2.3***	20.8 ± 2.9***	N.D.	N.D.	N.D.
Levomoramide	26.8 ± 1.8	27.4 ± 2.1	28.8 ± 1.9	29.0 ± 3.0	28.6 ± 2.3	27.6 ± 2.4	30.0 ± 2.5	N.D.	N.D.	N.D.
Methionine-enkephalin	22.0 ± 1.4	N.D.	N.D.	25.0 ± 4.6	27.6 ± 5.7	28.3 ± 4.1	32.0 ± 4.3**	34.3 ± 3.2**	37.0 ± 1.0**	36.0 ± 2.0**
II. Total T rosettes										
Morphine	68.0 ± 1.8	66.7 ± 2.2	68.0 ± 3.5	62.7 ± 3.3**	61.7 ± 2.4**	59.7 ± 1.5**	61.2 ± 1.5**	N.D.	N.D.	N.D.
Dextromoramide	69.7 ± 6.2	69.0 ± 3.0	67.5 ± 2.0	70.0 ± 5.0	67.5 ± 4.1	66.2 ± 3.6	68.7 ± 2.9	N.D.	N.D.	N.D.
Levomoramide	69.7 ± 6.2	67.3 ± 4.5	72.3 ± 5.5	69.0 ± 3.5	71.6 ± 1.5	70.0 ± 4.3	70.7 ± 4.2	N.D.	N.D.	N.D.
Methionine-enkephalin	69.0 ± 5.6	N.D.	N.D.	68.0 ± 2.6	69.0 ± 3.0	69.0 ± 5.3	71.0 ± 3.6	71.3 ± 5.7	67.0 ± 3.5	67.0 ± 3.6

[a] The data are the average results of five experiments done in duplicate. The statistical analysis compares the results between controls (no drugs) and the others: significant difference is marked by two asterisks ($p < 0.02$) or three asterisks ($p < 0.01$). The others are not significantly different.

[b] N.D., not done.

RESULTS

Table I shows the influence of 1-hr incubation with various drugs upon active and total T cell rosettes formation performed in five different instances with five different lymphocytes donors. In all five experiments, morphine decreased the percentages of active and total T rosettes with concentrations equal or higher than 10^{-10} M. Dextromoramide, with similar concentrations, also significantly decreased the percentage of active T rosettes in all the experiments. Levomoramide did not modify the percentage of active or total T rosettes: methionin-enkephalin significantly increased the percentages of active T rosettes at concentrations equal to or greater than 10^{-7} M in all five experiments. It did not affect the total T cell percentage. None of these drugs influenced the EAC percentages (results not shown).

Kinetics studies were established for the action of morphine and enkephalin by using the active T test. After 5 min of incubation with enkephalin or morphine, no effects were detected. After 15 min of incubation, the full inhibitory effect of morphine was already detected and was not modified with longer incubations. Similarly, the enhancing effect of methionin-enkephalin was already observed after 15 min of incubation and was not modified with longer periods of incubation.

Table II indicates the effect of naloxone upon rosette formation, in four different experiments, after a first incubation with morphine, dextromoramide or methionine-enkephalin at a concentration of 10^{-7} M. Naloxone completely inhibited the effect of these drugs. This reversibility was already complete at 10^{-8} M of naloxone for morphine and dextromoramide and at 10^{-6} M of naloxone for methionine-enkephalin. Naloxone by itself for 10^{-6} M, 10^{-7}, and 10^{-8} M did not modify the percentage of active or total T rosettes (active T rosettes in % ± S.D.: 24.6 ± 4.0 for 10^{-6} M, 24.8 ± 3.8 for 10^{-7} M, 24.2 ± 4.9 for 10^{-8} M. Total T rosettes in % ± S.D.: 68.4 ± 5.8 for 10^{-6} M, 68.6 ± 6.7 for 10^{-7} M, 71.8 ± 3.5 for 10^{-6} M). When the cells were first incubated with naloxone at 10^{-6} or 10^{-7} M, no reduction or augmentation of the percentages of active or total T rosettes were detected.

TABLE II

Percentages of active and total T rosettes (±S.D.) obtained after one incubation of cells with morphine, dextromoramide, or methionine-enkephalin at 10^{-7} M followed by second incubation without or with naloxone at 10^{-6}, 10^{-7}, or 10^{-8} M[a]

	Naloxone Concentration (M)			
	0	10^{-8}	10^{-7}	10^{-6}
I. Active T rosettes				
Morphine (10^{-7} M)	15.5 ± 2.6	22.7 ± 4.1**	23.2 ± 2.9**	24.5 ± 3.1**
Dextromoramide (10^{-7} M)	20.8 ± 2.9	26.8 ± 2.9***	27.0 ± 2.1***	27.6 ± 3.6***
Methionine-enkephalin (10^{-7} M)	32.0 ± 1.9	30.0 ± 4.3	27.3 ± 1.1	24.0 ± 2.6*
II. Total T rosettes				
Morphine (10^{-7} M)	61.2 ± 1.9	68.2 ± 2.9**	68.0 ± 2.4**	68.7 ± 3.3**

[a] The data are the average of four experiments done in duplicate. The statistical analysis compares the percentage obtained in the absence of naloxone or in its presence.

In three other experiments, the range of naloxone was extended from 10^{-12} M to 10^{-7} M after a first incubation of 1 hr with morphine or dextromoramide of 10^{-7} M by using the active T test. Significant reversibility of inhibition was seen up to a concentration of 10^{-10} M of naloxone. The following results were obtained: controls (no drug): 31.3 ± 1.5%, morphine 10^{-7} alone: 17.1 ± 1.0%, morphine 10^{-7} M plus naloxone 10^{-11} M: 19.7 ± 0.6% ($p < 0.05$ to controls), morphine 10^{-7} M plus

naloxone 10^{-10} M: 23.0 ± 5.6% (nonsignificantly different from controls), morphine 10^{-7} M plus naloxone 10^{-9} M: 26.0 ± 5.3% (nonsignificantly different from controls). Three similar experiments were performed with a range of various naloxone concentrations added after a first incubation with dextromoramide at 10^{-7} M by using the active T rosettes test. The reversibility of the inhibition was observed up to 10^{-8} M of naloxone. The results are as follows: controls (no drug): 29.3 ± 2.5%, dextromoramide 10^{-7} M alone: 16.7 ± 2.3%, dextromoramide 10^{-7} M plus naloxone 10^{-9} M: 20.3 ± 0.6%, ($p < 0.05$ compared to controls), dextromoramide 10^{-7} M plus naloxone 10^{-8} M: 28.3 ± 2.5% (not different from controls). When naloxone (10^{-7} to 10^{-12} M) and morphine (10^{-7} M) were incubated simultaneously for 1 hr before determining the percentages of active rosettes, the naloxone reversibility of inhibition was seen in all the three experiments performed. Reversibility was detected up to a concentration of 10^{-10} M of naloxone: controls: 29.7 ± 2.5%, morphine 10^{-7} alone: 18.0 ± 1.0% ($p < 0.05$ compared to controls), morphine 10^{-7} M plus naloxone 10^{-10} M: 21.3 ± 6.1% (nonsignificant from controls). These results were thus similar to the ones obtained where naloxone was incubated after morphine.

Similar three experiments were conducted for naloxone and dextromoramide, namely simultaneous incubation of the drugs for 1 hr and determination of the percentages of active T rosettes. Naloxone reversed the inhibition of dextromoramide 10^{-7} M up to a concentration of 10^{-9} M: controls: 30.7 ± 2.5%, dextromoramide 10^{-7} M: 18.0 ± 1.7%, dextromoramide 10^{-7} M plus naloxone 10^{-9} M: 21.0 ± 1.0% ($p < 0.05$ compared to controls), dextromoramide 10^{-7} M plus naloxone 10^{-8} M: 23.7 ± 7.2% (nonsignificantly different from controls). Naloxone added simultaneously during the 15-min incubation experiments abolished the effects of morphine or methionin-enkephalin.

DISCUSSION

The present observations indicate that morphine and methionine-enkephalin influence the rosette formation between human T lymphocytes and SRBC (Table I). Since methionine-enkephalin could be degraded after 1 hr in incubation time at 37°C, shorter incubation times were also investigated. The effect was already observed after 15 min of incubation with the drug. The specificity of this phenomenon is proven by its complete reversibility in the presence of similar concentrations of naloxone, an antagonist of these drugs (Table II). Furthermore, prior or simultaneous incubation of the lymphocytes with appropriate concentrations of naloxone completely prevents the modification of rosette formation induced by morphine or methionine-enkephalin. Other antagonists, like diprenorphine or nalorphine, were not used in these experiments since they have the disadvantage of also possessing some agonist activities that would have made difficult the interpretation of the results.

Finally, dextromoramide, an active analog of morphine, has the same effects as morphine and is similarly reversed by naloxone, whereas levomoramide, the inactive enantiomere, does not influence rosette formation (8). This confirms the stereospecificity of this phenomenon.

On the basis of the results obtained with peripheral blood lymphocytes, it is proposed that normal human blood T lymphocytes bear surface receptor-like structures for morphine and methionine-enkephalin. Except in the case of morphine, the influence upon rosette formation was only detected with the active rosette test. This is readily explained by the fact that the active T test is performed under suboptimal conditions for rosette formation (temperature of 37°C, low SRBC per lymphocyte ratio, and short incubation time between SRBC and lymphocytes). Therefore, the active T test is expected to be more easily influenced than the total T test performed in optimal technical conditions (7).

The consistently opposite effects of morphine and methionine-enkephalin on rosette formation are unusual and thus more difficult to explain. It may perhaps imply the presence of two receptors: one for morphine and some synthetic analogues like dextromoramide and one for methionine-enkephalin. Even if no opposite effects of morphine and methionine-enkephalin have been described in other systems, the existence of different kinds of opioid receptors has been confirmed in isolated organ preparations. The μ-receptors more sensitive to morphine would be prodominant in guinea-pig ileum whereas the δ more sensitive to enkephalins would be predominant in the mouse vas deferens (9). The μ receptors would be also more sensitive to the reversal by naloxone. In the present study, one can notice that the action of morphine was more easily reversed by naloxone than that of methionine-enkephalin (Table II).

The findings of receptors on T lymphocytes for drugs and substances known to bind to nervous cells may provide further insight into the relations between the immune system and the central nervous system. Such links may be of utmost importance in various disease states like slow virus infections, multiple sclerosis, and perhaps neurotic and psychotic disorders where immune mechanisms may be involved (10–12). The search for a role of the enkephalins may, among many, be that of mediator between the central nervous system and the immune system.

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