

## POTENTIAL EFFECTS OF PSYCHOACTIVE DRUGS ON PRIMARY IMMUNE RESPONSES TO SRBC IN MICE

Amaia Arregi\*, Larraitz Garmendia\*, María Luisa Andrade\*\*,  
José R. Sánchez-Martín\* y Paul F. Brain\*\*

\* Universidad del País Vasco, \*\* University College of Swansea (Great Britain)

The impact on primary hemagglutinin titres in response to a challenge with a suspension of sheep red blood cells of treating male and female mice with naloxone or chlordiazepoxide was assessed. Naloxone increased total antibody titres in females but reduced it in males. This mu antagonist increased mercaptoethanol-resistant hemagglutinin titres in both males and females, with males showing the greater increase. Chlordiazepoxide reduced both total and mercaptoethanol-resistant antibody titre in both males and females, males showed a more marked suppression. Interestingly, naloxone is anxiogenic and chlordiazepoxide anxiolytic. The data suggest that treatment with psychoactive drugs is likely to influence disease resistance in organisms, data reminiscent of repeated claims that psychological factors influence immunoresponsiveness.

*Efectos potenciales de sustancias psicoactivas sobre la respuesta primaria inmune a SRBC en ratones.* Se realizó un experimento en el que se valoró el efecto del tratamiento con naloxona y clordiazepóxido sobre la respuesta inmunitaria primaria de ratones machos y hembras. La actividad inmunitaria se estimó mediante la medida del título de anticuerpos en un test de hemaglutinina en respuesta a la aplicación de una suspensión estándar de glóbulos rojos de carnero. La naloxona aumentó el título total de anticuerpos en hembras y los disminuyó en machos. Este antagonista de los receptores opiáceos Mu aumentó los títulos de hemaglutinina resistentes al mercaptoetanol tanto en machos como en hembras, aunque los machos presentaron una mayor supresión. Los datos sugieren que el tratamiento con fármacos psicoactivos, en nuestro caso un anxiógeno como la naloxona y un anxiolítico como el clordiazepóxido, puede influir sobre la resistencia de los organismos a las enfermedades. Cada vez un número mayor de datos indican que los factores psicológicos influyen sobre la capacidad de respuesta inmunitaria.

Psychological factors clearly influence immune responses in humans (Locke, 1982; Irwin, Daniels, Bloom, Smith, &

Weiner, 1987; Temoshok, 1987) and other animals (Borysenko & Borysenko, 1982; Griffin, 1989). Relationships between the nervous system and the immune system may be mediated indirectly via the hypothalamic-hypophysial axis or directly through the autonomic nervous system. In addition, there are receptors for a variety

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Correspondencia: Dra. Amaia Arregi Aguirre  
Area de Psicobiología. Facultad de Psicología  
Universidad del País Vasco  
Avda. Tolosa, 70. 20009 San Sebastián (Spain)

of ligands associated with nervous system (neurotransmitters and hormones) located on cells that are components of the immune system (Jankovic, 1989; Rabin, Cohen, Ganguli, Lysle, & Cunnick, 1989; Khansari, Murgo, & Faith, 1990; Blalock, 1992; Jancovick & Radulovic, 1992).

It consequently seemed logical to examine the effects of psychoactive drugs on the immune system's responses. A wide variety of neural opioid receptors are closely involved in behavioural changes (Rance, 1983). The relationship between endogenous morphine and stress suggests that endorphins and enkephalins influence emotionality (Rossier & Chapouthier, 1983). The most commonly-used opioid antagonist is naloxone, which specifically blocks mu receptors at low doses but at higher doses it also influences kappa and delta receptors, as well as changing gabaergic activity (Dingledine, Iversen, & Breuker, 1978). This drug dose-dependently increases sensitivity to shock punishment on a food reinforced operant in the rat (Young, 1980). Under certain circumstances, morphine restores the punishment response in pigeons to a level similar to that seen after application of benzodiazepines. Moreover, those results mirror the anxiolytic effects of a morphine shown in humans (Iversen & Iversen, 1981). Lower doses of naloxone than those needed to increase the punishment effect, reverse the tolerance to punishment produced by diazepam administration (Soubrie, Jobert, & Thiebot, 1980).

Benzodiazepines have been characterized as efficient anxiolytics. Their therapeutic effect appears mainly mediated by interactions with receptors of the gabaergic system (Tollman, Skolnik, & Gallagher, 1980; Olsen, 1982; Paul, 1986; Doble & Martin, 1992). Many studies on the effects of benzodiazepines on anxiety have been carried out using experimental ani-

mals. Animals appear less fearful, and more ready to carry out activities for which they have been punished, after treatment with these compounds (Treit, 1985; Iversen & Iversen, 1981). Chlordiazepoxide (CDP) is a classical benzodiazepine, widely-used in this type of research, which has a strong anti-punishment effect (Iversen & Iversen, 1981).

The present study investigated the effects of naloxone and CDP on the primary immune response in male and female mice. Given the capacity of these drugs to affect emotional states, an influence on the immune response seemed likely. There are clear sex differences in emotionality, measured by behaviour in open field (defecation and ambulation) and, as response to novel situations such as stress and intense population density (Gray, 1971; Steenbergen, Heinsbroek, Van Hest, & Van de Poll, 1990; Johnston & File, 1991). The effects of sex on the immune response were consequently also examined.

## Materials and methods

### *Animals*

Sixty male and sixty female AP albino mice, from a stock obtained from ICI Pharmaceuticals, Macclesfield, U.K. were used in the study. The subjects were reared under highly controlled conditions at the animal facilities of University College of Swansea, U.K. (described in Brain, McAllister, & Walmsley 1989). All animals lived under a reversed lighting schedule (white lights on from 2200 to 1000 h local time).

### *Drugs*

Both naloxone hydrochloride and CDP were obtained from Sigma Chemical Company Ltd., Fancy Rd., Poole, Dorset,

BH17 7NH, England. The materials were made up freshly in physiological (0,85%) saline.

*Treatments*

All animals were injected subcutaneously (sc) each day for a 7 day period, two hours after the beginning of the dark period. Categories of males and females (n=10) were given daily injections of either 1 or 2 mg/kg of naloxone or 5 or 10 mg/kg of CDP. Control categories received physiological saline. Five days after the end of this treatment, subjects received an antigenic challenge (see below).

*Immunological Methods*

Sheep red blood cells (SRBC) from Flow Laboratories (Irvine, Scotland) were washed three times with physiological saline and made up to a 20% suspension in saline (there were approximately 10<sup>9</sup> cells/ml). The standard antigenic challenge consisted of a 0,1 ml intraperitoneal (ip) injection of this suspension (10<sup>8</sup> cells). Blood was subsequently collected from subjects by cardiac puncture 5 days after challenge and it was allowed to clot at room temperature before being centrifuged at 3,000 rpm. The resulting sera were collected and incubated for complement inactivation in a water bath at 56<sup>0</sup>C for 30 min. Serum titrations were carried out in polystyrene trays (type 2-45128A, Nunc Gibco, Life Technologies, Paisley, Scotland) using serial two-fold dilutions, with 0,85% saline and Tris/HCl buffer solution at a pH of 7,4. After incubating the sera at 37 <sup>0</sup>C, dilutions containing total hemagglutinins were recorded and immune response assessed in terms of mean log<sub>2</sub> titres for each category. Serum was also titrated after treatment with 2-mercaptoethanol (Sigma Chemicals Company, LTD.,

Fancy Rd., Poole, Dorset, BH17 7NH, England). MER-resistant antibody is the 7S (Sredberg units) variety, whereas total antibody titre gives the 7S and 12S values (combined). The primary immune response is largely in terms of 12S antibody (Brayton & Brain, 1974).

**Results**

Mean log<sub>2</sub> antibody titres for total and mercaptoethanol-resistant (MER-resistant) hemagglutinins together with ANOVA comparisons between categories are given in Tables 1 and 2, respectively. Figures 1 to 4 represent the effects of naloxone and CDP doses on total and MER-resistant antibody production.

*Naloxone*

The total hemagglutinin measure showed a significant interaction between drug and sex (p< 0.04). The 10 mg/kg dose affected males and females in different ways.

Table 1 Naloxone's effects on median total and MER-resistant hemagglutinin titres in male and female mice						
SEX	Total HG			MER-resistant HG		
	Control	1mg/kg	10 mg/kg	Control	1mg/kg	10 mg/kg
Female	3,30	3,10	4,10	1,15	1,80	1,78
Male	3,65	3,89	1,86	1,50	2,44	2,00
ANOVA						
		Total HG		MER-resistant HG		
		F	Signif	F	Signif	
Sex		0,060	0,80	5,200	0,02	
Naloxone		0,175	0,84	8,719	0,00	
Sex x Naloxone		3,506	0,04	0,404	0,70	

In males there was a drastic reduction of antibody titres, but females showed an increased response. MER-resistant antibody showed a significant increase after

treatment with naloxone ( $p < 0.0001$ ). Both doses (1 mg/kg and 10 mg/kg) differed significantly from the control group ( $p < 0.001$  and  $p < 0.009$ ). There were also significant effects of sex ( $p < 0.02$ ) on this measure, males having higher antibody titres than females in the different groups.

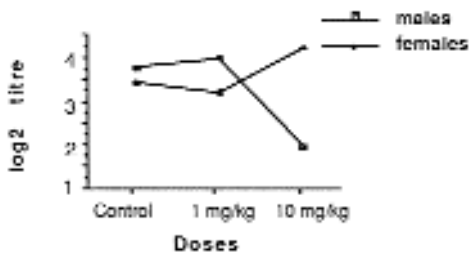


Figure 1. Impact of naloxone on total hemagglutinin titre in male and female mice.

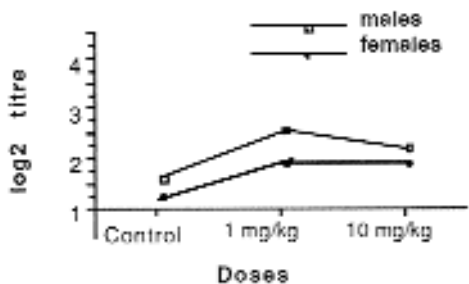


Figure 2. Impact of naloxone on MER-resistant hemagglutinin titre in male and female mice.

CDP

A significant effect on the total hemagglutinin titre of mice was observed when subjects were treated with CDP ( $p < 0.03$ ). The 10 mg/kg dose produced a significant reduction in antibody titre compared with controls ( $p < 0.01$ ). There were no sex differences in total hemagglutinin production. CDP tended to reduce

the MER-resistant antibody titre ( $p < 0.07$ ). Nevertheless, when median values for the groups were compared, there was a significant difference between the 5 and 10 mg/kg doses ( $p < 0.0001$ ) and between the 5 mg/kg and control ( $p < 0.0001$ ). A reduction in the MER-resistant antibody concentration was observed with the 5 mg/kg dose but not with the 10 mg/kg dose. There was a significant difference between sexes in the MER-resistant hemagglutinin response ( $p < 0.05$ ), with males producing more antibody than females.

*Table 2*  
Cordiazepoxide's effects on total and MER-resistant hemagglutinin titres in male and female mice

SEX	Total HG			MER-resistant HG		
	Control	1mg/kg	10 mg/kg	Control	1mg/kg	10 mg/kg
Female	3,30	3,10	2,50	1,15	1,10	1,00
Male	3,65	3,89	2,30	1,50	1,00	1,22

ANOVA	Total HG		MER-resistant HG	
	F	Signif	F	Signif
Sex	0,000	1,00	3,795	0,05
Naloxone	3,531	0,03	2,794	0,07
Sex x Naloxone	0,573	0,57	1,543	0,2

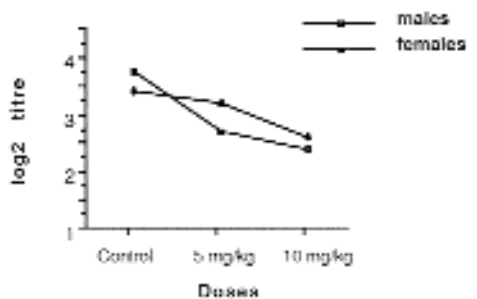


Figure 3. Impact of chlordiazepoxide on total hemagglutinin titre in male and female mice.

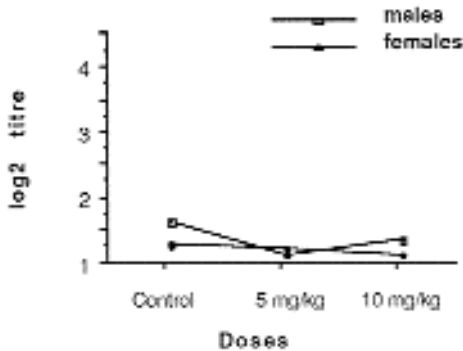


Figure 4. Impact of chlordiazepoxide on MER-resistant hemagglutinin titre in male and female mice.

### Discussion

Treatment with naloxone at both doses (1 and 10 mg/kg) increased antibody titre in response to SRBC inoculation on the MER-resistant hemagglutinin test. This effect on immunopotential is reminiscent of that reported by Inostroza, Teschemacher, & Mueller-Eckhardt, (1987), Jankovic & Maric (1987) and Mediratta, Das, Gupta, & Sen, (1988), which all suggest that naloxone antagonizes the immunosuppressive effects of endorphins. This immunosuppression has also been observed both on cellular (Shavit, Lewis, Terman, Gale, & Liebeskind, 1986; Maric & Jankovic, 1987; Gabrilovac, Antica & Osmak, 1992; Freier & Fuchs, 1994) and humoral (Johnson, Smith, Torres, & Blalock, 1982; Mediratta et al., 1988) immune responses.

Naloxone's effects on MER-resistant hemagglutinin differ from those on total hemagglutinin, suggesting that this drug affects those two types of hemagglutinins in different ways. This difference was sex-dependent. In the case of the MER-resistant hemagglutinin a quantitative difference on the antibody response was observed, while in the total hemagglutinin titre a

sex-linked qualitative difference in response to naloxone was found, with a significant sex-drug interaction.

Sex differences in the immune system have also been reported by Globe & Kopka (1973) and Rabin et al. (1988) and it is generally accepted that males are more vulnerable to the effects of stress than females (Strausser, Fiore, & Belisle, 1984; McFarland & Bigley, 1989), although existing results are not conclusive.

In the present experiment, a higher production of MER-resistant hemagglutinin was evident in males, independently of the drug used. This dimorphism was also observed in the control groups.

The present results also show that CDP application affects the immune system. This drug depressed the antibody response in both tests, although the overall effect observed on MER-resistant hemagglutinin did not quite reach significance. In this test, the 5 mg/kg dose produced a significant reduction on the antibody production compared to both control group and 10 mg/kg group. Pericic, Manev, Boranic, Poljak-Blazi, & Lehic (1987), reported that another benzodiazepine (Diazepam) also has an immunosuppressive effect on spleen plaque-forming cell production in rats. It has also been observed the sex differences in MER-resistant hemagglutinin (but not total hemagglutinin) titre were evident in this study. Relationships between the immune response and benzodiazepine receptors have been suggested by Arora, Hanna, Paul, & Skolnick (1987). Receptors for these drugs are located in regions of the cerebral cortex which are concerned with both anxiety mechanisms and the immune response.

Essentially the present results confirm that naloxone and CDP have opposite effects on antibody production. Several data show these drugs alter lymphocytes T population (Arora, Hanna, Paul & Skolnick,

1987; Inostroza, Teschemacher & Mueller-Eckhardt, 1987; Manfredi, Sacerdote, Vianchi, Locatelli, Veljic-Radulovic & Panerai, 1993). A subset of lymphocytes cells, helper - T cells, influence antibody production; the B cells' response to antigens is totally dependent on these cells. This mechanism could be the responsible of the effects of both sustances on immune primary response. There is evidence that endogenous opiates decrease antibody response to T dependent antigens (SRBC) and this effect was reversed by naloxone (Inostroza, Teschemacher & Mueller-Eckhardt, 1987; Manfredy, Sacerdote, Bianchi, Locatelli & Veljic-Radulovic, 1993).

In the other hand, as naloxone and CDP affect anxiety in opposite ways (naloxone is anxiogenic whereas CDP is anxiolytic) the opposite effects on antibody production seems a logical finding. The different

anxiety states generated by these drugs (with a possible gabaergic involvement and productions of endogenous opioids), could be the responsible for the varied effects of these psychoactive compounds on the immune response. The regions of the cerebral cortex that influence anxiety mechanisms also alter the immune system. The data suggest that emotional states are likely to influence immune responsiveness and that there are likely to be immunological sequelae of clinical treatment with psychoactive compounds. The potential impact of psychoactive compounds on disease resistance must, for example, be of great interest to AIDS researchers, as AIDS sufferers are frequently treated for depression. It is notable that males and females appear to show differing responses (in terms of immune capacity) to these compounds.

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