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# Comparison between the effects of σ receptor ligand JO 1784 and neuropeptide Y on immune functions

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**Abstract**

Recent evidence suggests that σ receptor ligands and neuropeptide Y may act through the same pathways to modulate centrally mediated immune function. The present study demonstrated that both the σ receptor ligand igmesine: (+)-*N*-cyclopropylmethy-*N*-methyl-l, 4-diphenyl-l-yl-but-3-en-1-ylamine, hydrochloride (JO 1784) ($10^{-7}$ and $10^{-5}$ M) and neuropeptide Y ($10^{-9}$ and $10^{-7}$ M) in vitro significantly reduced neutrophil phagocytosis and decreased mitogen stimulated lymphocyte proliferation. By contrast, central administration of JO 1784 (0.5 and 5 μg/5 μl) significantly reduced the activity of neutrophil phagocytosis, but enhanced lymphocyte proliferation without changing the serum concentration of corticosterone. Neuropeptide Y ($10^{-9}$ and $10^{-7}$ M), following intracerebroventricular infusion, also decreased the neutrophil response, but significantly raised the corticosterone concentration. These results indicate that different mechanisms (involving various neurotransmitters and their receptors, changes in the activity of the hypothalamic–pituitary–adrenal axis, or σ receptor subtypes) may be involved in the central effects of JO 1784 and neuropeptide Y. © 1998 Elsevier Science B.V.

*Keywords:* σ Receptor ligand; JO 1784; Neuropeptide Y; Neutrophil phagocytosis; Lymphocyte proliferation

## 1. Introduction

σ Receptors have been defined as non-opiate, non-dopaminergic and non-phencyclidine (PCP) receptors (Walker et al., 1990; Quirion et al., 1992). Most σ receptor ligands produce behavioural excitation, elevate dopaminergic function and interact with the NMDA receptors in the central nervous system (CNS) (Sagratella et al., 1991; Zhang et al., 1993). These effects are similar to those caused by phencyclidine (PCP) (Conteras et al., 1988; Monnet et al., 1992a). Both σ receptor ligands and PCP receptors have been found to protect the brain from neurotoxicity by inhibition of glutamate-activated cGMP formation (Lesage et al., 1995; Lockhart et al., 1995). In the periphery, it has been reported that σ receptor ligands may exert immunosuppressive and endocrine effects directly through actions on the pituitary gland and other target organs (Sagratella et al., 1991; Wolfe and De Souza, 1993). For example, haloperidol and 1,3-di-(*o*)-tolylguanidine (DTG) suppress in vitro murine nature killer (NK) cell activity, while PCP, DTG and (+)-pentazocine significantly reduce lectin-induced lymphocyte proliferation (Carr et al., 1992). Some σ receptor ligands have been also shown to elevate plasma corticosterone concentration via an action on serotonin (5-hydroxytryptamine, 5-HT) receptors (Gudelsky and Nash, 1992).

JO 1784 (igmesine: (+)-*N*-cyclopropylmethy-*N*-methyl-l, 4-diphenyl-l-yl-but-3-en-1-ylamine, hydrochloride) is a novel and potent ligand for σ sites (Roman et al., 1990). In addition, JO 1784 has anti-amnesic activity by increasing acetylcholine release and enhancing NMDA induced neuronal activation of CA3 hippocampal cells (Earley et al., 1991; Monnet et al., 1991). However, the central role of σ receptor ligands in immune modulation and the effects of JO 1784 on cellular immunity are unclear.

The effects of neuropeptide Y on the immune and endocrine systems and brain have been extensively investigated (Dumont et al., 1992; Friedman et al., 1995; Song et al., 1996). Most neuropeptide Y studies in vitro showed that the peptide inhibits immunocyte functions, such as NK cell cytotoxicity, macrophage activity and neutrophil chemotaxis (Nair et al., 1993; Dureus et al., 1993).

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We have previously reported that central administration of a low dose of neuropeptide Y (0.5 nM) did not affect lectin-stimulated lymphocyte proliferation or the differential white blood cell count in intact rats but did increase the serum corticosterone concentration and reversed the suppressed lymphocyte function in the olfactory bulbectomized rat model of depression (Song et al., 1996).

$\sigma$ receptors are distributed in the same brain regions and in the adrenal gland and leukocytes as neuropeptide Y (Adrian et al., 1983; Tam and Cook, 1984; Heilig et al., 1988; Su et al., 1990; Dumont et al., 1992). There it evidence that $\sigma$ receptor ligands and neuropeptide Y interact. Thus it has been found that the sigma ligand haloperidol increased neuropeptide Y immunoreactivity in the locus coeruleus (Smialowska and Legutko, 1992). In addition, central administration of neuropeptide Y and JO 1784 reverse corticotrophin-releasing factor (CRF)-induced colonic motor activity (Junien et al., 1991; Gue et al., 1992). These effects of JO 1784 and neuropeptide Y may be associated with their actions on $\sigma$ receptors and Gi protein linked second messengers (Junien et al., 1991; Bueno et al., 1992). However, so far few investigators have studied the effects of $\sigma$ receptor ligands and NPY on the immune functions, in vivo and in vitro. The purpose of this study was therefore to compare the effects in vivo and in vitro of $\sigma$ receptor ligands JO 1784 and neuropeptide Y on neutrophil phagocytosis, lymphocyte proliferation and serum corticosterone concentration. By comparing the effects of the drugs on these processes it should be possible to determine whether JO 1784 and NPY have qualitatively effects that may implicate similar intracellular changes involving $\sigma$ receptors.

## 2. Materials and methods

### 2.1. Animals

Adult male Sprague–Dawley rats (250–280 g) were obtained from Harlan Olac, Blackthorn, Bicester, UK and maintained on a 12 h light and 12 h dark cycle at room temperature $21 \pm 1$°C. Water and food were available ad libitum.

### 2.2. Reagents

Neuropeptide Y (porcine form) was obtained from Sigma Chemical Co., London and JO 1784, PCP, DTG and haloperidol were obtained from Jouveinal Research Institute, Fresnes, France.

For the i.c.v. administration, JO 1784 and neuropeptide Y were dissolved in sterile physiological saline. For in vitro study, DTG, PCP, haloperidol, JO 1784 and neuropeptide Y were dissolved in RPMI-1640 medium (Gibco Life Technologies, UK) for incubation with lymphocytes. JO 1784 and neuropeptide Y were dissolved in Hank's balance salt solution (HBSS) (Gibco Life Technologies, UK) for incubation with neutrophils.

The lectins, phytohemagglutinin-P, concanavalin A, pokeweed mitogen, luminol and zymosan were purchased from Sigma Chemical Co., London; [$^3$H]thymidine was purchased from Amersham Co., London.

### 2.3. Procedures

Following intraventricular cannulation, rats were allowed to recover for two weeks. They were then assigned to seven acute i.c.v. treatment groups of 8 rats per group: (1) control (for neuropeptide Y treatment) infused with 5 $\mu$l saline; (2) 5 $\mu$l of neuropeptide Y at $10^{-9}$ M; (3) neuropeptide Y at $10^{-7}$ M; (4) control (for JO 1784 treatment) infused with 5 $\mu$l saline; (5) JO 1784 at 0.1 $\mu$g/5 $\mu$l; (6) JO 1784, 0.5 $\mu$g/5 $\mu$l and (7) JO 1784, 5 $\mu$g/5 $\mu$l. These concentrations were chosen from the results of previous experiments by Heilig et al. (1990), Gue et al. (1992), Nagain et al. (1995) and Song et al. (1996). Animals were habituated to i.c.v. infusion by handling and manipulation of the cannulae daily for a week prior to the treatment. In all treatments 5 $\mu$l of either saline, JO 1784 or neuropeptide Y were infused by gravity as described by Song and Leonard (1995). Following acute infusion over a period of 40–45 min, blood samples were taken into heparinized syringes by cardiac puncture under diethyl ether anaesthesia (Song and Leonard, 1994a, 1995). These blood samples were used for neutrophil phagocytosis and lymphocyte proliferation. Then the animals were decapitated and the trunk blood was collected. The serum from trunk blood was used to determine the concentration of corticosterone or to opsonise the zymosan.

To examine whether JO 1784 in vitro has similar effects as other $\sigma$ receptor ligands, blood samples from eight intact rats were taken by cardiac puncture to separate lymphocytes. DTG, PCP, haloperidol and JO 1784 were incubated with these lymphocytes.

To compare the in vitro effects of JO 1784 and neuropeptide Y on immune cellular functions, blood samples from eight intact rats were taken by cardiac puncture to separate neutrophils and lymphocytes. Purified neutrophils or lympocyte from each rat was divided into two equal volumes: one volume was incubated with RPMI-1640 or HBSS as control and the other with different concentrations of neuropeptide Y or JO 1784.

### 2.4. Surgery

Rats were anaesthetised with tribromoethanol (250 mg/kg i.p. (Van Riezen and Leonard, 1990) and placed in a sterotaxic apparatus. Cannulae were located stereotaxically over the lateral cerebral ventricle as described in detail elsewhere (Song and Leonard, 1995; Song et al., 1996). The needle was extended 1 mm beyond the tip of the guide cannula into the lateral ventricle.

### 2.5. Preparation of neutrophils and lymphocytes

Plasma gel (2–3 ml) (Hausmann Lab., St. Galley, Switzerland) was added to each blood sample (5–6 ml) and mixed. After leaving for 30 min at room temperature, the mixture of neutrophils and lymphocytes in the supernatant were layered onto a Nycoprep gradient (Nycomed AS Oslo, Norway) and centrifuged at $600 \times g$ for 20 min at 4°C. Following centrifugation, two distinct bands of white cells were obtained. The upper layer contained the lymphocytes and the lower the neutrophils. The purity of these fractions was approximately 98% as assessed by microscopic examination. Neutrophils were washed three times using HBSS and lymphocytes were washed three times with RPMI-1640. The number of neutrophil cells was adjusted to $10^6$ cells/ml in HBSS for the determination of phagocytic activity. The number of lymphocytes were adjusted to $2 \times 10^6$ cells/ml for determination of the mitogen stimulated lymphocyte proliferation.

### 2.6. Neutrophil phagocytosis and mitogen stimulated lymphocyte proliferation

The neutrophil phagocytosis was performed using a modification of the method of O'Neill and Leonard (1990). Briefly, the trunk blood was centrifuged at $700 \times g$ for 10 min and the serum then removed for the opsonisation of the zymosan. The phagocytic activity of neutrophils was measured in a luminometer. A 200 $\mu$l aliquot of neutrophil cells was mixed with 200 $\mu$l of $10^{-4}$ M luminol and placed in the light-sealed measuring chamber of the luminometer. The chemiluminescence response (in mV) stimulated by engulfment of the zymosan particles by neutrophils was recorded for 25 min. The onset of phagocytosis was expressed in s (Hosker et al., 1989). For the in vitro study, $10^{-9}$ or $10^{-7}$ M neuropeptide Y (Soder and Hellstrom, 1987; Hafstrom et al., 1993) and $10^{-9}$, $10^{-7}$ or $10^{-5}$ M JO 1784 was incubated with neutrophils for 40–45 min, respectively (Song and Leonard, 1994a).

Lymphocyte proliferation was performed in triplicate as previously described (Song and Leonard, 1994a,b). The lymphocyte suspension was placed in each well of 96 well microtiter plate containing 0.2 ml of RPMI-1640 medium supplement with penicillin (2%) and heat-inactivated foetal calf serum (10%). To each well, one of the following reagents was added: RPMI-medium for background activity, optimal concentration of the lectins: phytohemagglutinin, 10 $\mu$g/ml; concanavalin A, 2 $\mu$g/ml and pokeweed mitogen, 10 $\mu$g/ml (Song et al., 1996). For the in vitro comparative study between JO 1784 and neuropeptide Y, neuropeptide Y ($10^{-9}$ or $10^{-7}$ M) and JO 1784 ($10^{-9}$, $10^{-7}$ or $10^{-5}$ M) were added to the wells containing the medium, phytohemagglutinin, concanavalin A or pokeweed mitogen (Carr et al., 1992). For the comparative study between 'more classical' $\sigma$ receptor ligands and JO 1784, DTG, PCP, haloperidol and JO1784 at $10^{-5}$ M concentration (Sonders et al., 1988; Carr et al., 1991, 1992) were added to 96 well plate containing above three lectins. The cultures were then incubated for 52 h at 37°C in a 5% $CO_2$ atmosphere. After addition of tritiated thymidine (0.5 $\mu$Ci/well) for 18 h, the cells were harvested (Dynatech Multimash-2000), Germany). The tritiated thymidine uptake was then measured in a scintillation counter (Beckman, LS-7000, USA). Mean scintillation counts per min (cpm) were determined.

### 2.7. Assay of corticosterone

Blood samples were allowed to clot for at least 30 min at room temperature. These samples were centrifuged at $800g$ for 10 min and the resultant supernatant was removed. Serum corticosterone concentrations were determined by a modification of the method of Glick et al. (1964). Briefly, samples were mixed in 600 $\mu$l chloroform for 15 s. 500 $\mu$l of the chloroform phase was transferred into a tube containing 400 ml sulphuric acid:ethanol (32.5:17.5) and mixed for 15 s. The samples were then kept in the dark for 45 min. A 300 $\mu$l aliquot from the lower acid phase was removed and fluorescence measured in a specrophotofluorimeter. Results are expressed as $\mu$g/dl of serum.

### 2.8. Statistics

For the in vivo study, the differences between control and drug treatment groups were analysed by repeated analysis of variance (ANOVA) followed by the least significant difference (LSD) (Winer, 1971). The results are expressed as mean $\pm$ S.E.M. The Wilcoxon match-pairs signed-ranks test was used to analyse the responses of neutrophil phagocytosis and lymphocyte proliferation in vitro with each sample acting as its own control. These results are expressed as median and inter quartile range (Winer, 1971).

## 3. Results

### 3.1. Suppressive effects of $\sigma$ receptor ligands on lymphocyte proliferation in vitro

Table 1 shows that in vitro $\sigma$ receptor ligands DTG, PCP and haloperidol at dose $10^{-5}$ M significantly suppress phytohemagglutinin, concanavalin A and pokeweed-stimulated lymphocyte proliferation. JO 1784 at the same dose also significantly decreases these three mitogen-stimulated lymphocyte proliferation (Table 1).

### 3.2. Immunosuppressive effects of JO 1784 and neuropeptide Y in vitro

After incubation of JO 1784 with neutrophils for 45 min, the peak height of phagocytosis was significantly

Table 1
The effects of $\sigma$ receptor ligands on mitogen stimulated lymphocyte proliferation in vitro in the rat

| [$^3$H]-uptake (cpm $\times 10^3$) | Control (medium) | DTG ($10^{-5}$ M) | Haloperidol ($10^{-5}$ M) | PCP ($10^{-5}$ M) | JO1784 ($10^{-5}$ M) |
|---|---|---|---|---|---|
| PHA (10 $\mu$g/ml) | 40.86 (32.28–49.16) | 32.20 (23.55–40.81)[a] | 31.54 (24.06–39.75) | 30.76 (23.72–39.72)[a] | 18.26 (12.69–22.35)[b] |
| Con A (2.0 $\mu$g/ml) | 69.16 (38.67–85.01) | 49.77 (15.59–56.16)[a] | 47.96 (15.23–58.36)[a] | 47.12 (15.76–60.39)[a] | 42.48 (24.97–56.75)[b] |
| PWM (10 $\mu$g/ml) | 20.91 (14.09–27.02) | 14.02 (8.56–23.83)[a] | 1867 (9.34–26.79) | 13.41 (7.98–21.42)[a] | 10.97 (5.34–19.30)[b] |

Results are expressed as median (inter quartile range).
Lymphocyte number = $2 \times 10^6$.
[a] $P < 0.05$.
[b] $P < 0.01$ compared to controls.
$n = 8$.

Table 2
The inhibitive effect of $\sigma$ receptor ligand JO 1784 on neutrophil phagocytosis and mitogen stimulated lymphocyte proliferation in vitro in the rat

| | Control (medium) | JO 1784 ($10^{-9}$ M) | JO 1784 ($10^{-7}$ M) | JO1784 ($10^{-5}$ M) |
|---|---|---|---|---|
| Phagocytosis peak height (mV) | 24.46 (20.21–29.32) | 22.79 (17.51–27.01) | 17.24 (13.22–25.49)[a] | 12.37 (6.63–17.61)[b] |
| Time of onset (s) | 49.44 (43.57–58.82) | 49.63 (44.31–62.48) | 56.58 (49.39–65.77)[a] | 69.15 (61.72–78.21)[a] |
| Proliferation: PHA (10 $\mu$g/ml) | 42.25 (32.54–48.24) | 34.20 (24.55–40.81) | 24.24 (16.67–36.62)[a] | 17.37 (13.45–23.31)[b] |
| Con A (2.0 $\mu$g/ml) | 67.18 (37.61–86.11) | 57.37 (31.53– 85.24) | 47.13 (15.77–60.41)[a] | 44.03 (21.11–66.65)[b] |
| PWM (10 $\mu$g/ml) | 21.43 (14.98–28.36) | 18.96 (3.12–28.29) | 14.40 (3.9–23.10)[a] | 11.41 (5.68–21.30)[b] |

Results are expressed as median (inter quartile range).
Neutrophil number = $10^6$, lymphocyte number = $2 \times 10^6$.
[a] $P < 0.05$.
[b] $P < 0.01$ compared to controls.
$n = 8$.

Table 3
The effect of neuropeptide Y on neutrophil phagocytosis and mitogen stimulated lymphocyte proliferation in vitro in the rat

| | Control (medium) | NPY ($10^{-9}$ M) | NPY ($10^{-7}$ M) |
|---|---|---|---|
| Phagocytosis peak height (mV) | 21.87 (18.33–27.32) | 19.74 (16.49–23.01)[a] | 16.38 (14.63–19.74)[b] |
| Time of onset (s) | 58.44 (52.57–64.12) | 58.63 (54.31–66.47) | 64.79 (59.82–70.23)[a] |
| Proliferation: PHA (10 $\mu$g/ml) | 39.15 (30.22–46.24) | 33.12 (19.60–40.72)[a] | 28.87 (23.45–44.31)[a] |
| Con A (2.0 $\mu$g/ml) | 69.16 (38.69–85.01) | 58.24 (27.58–74.11)[a] | 47.89 (22.33–60.86)[b] |
| PWM (10 $\mu$g/ml) | 20.91 (14.09–27.02) | 19.76 (15.12–28.29) | 14.56 (12.34–20.31)[a] |

Results are expressed as median (inter quartile range).
Neutrophil number = $10^6$; Lymphocyte number = $2 \times 10^6$.
[a] $P < 0.05$.
[b] $P < 0.01$ compared to controls.
$n = 8$.

Table 4
The effects of i.c.v. administration of $\sigma$ receptor ligand JO 1784 on neutrophil phagocytosis and mitogen stimulated lymphocyte proliferation in the rat

| | Control (medium) | JO 1784 (0.1 $\mu$g/5 $\mu$l) | JO 1784 (0.5 $\mu$g/5 $\mu$l) | JO1784 (5.0 $\mu$g/4 $\mu$l) |
|---|---|---|---|---|
| Phagocytosis peak height (mV) | 39.44 ± 4.35 | 33.77 ± 7.85 | 23.55 ± 5.5[a] | 19.58 ± 4.55[b] |
| Time of onset (s) | 62.86 ± 6.44 | 78.75 ± 8.33 | 81.43 ± 7.05 | 84.29 ± 7.51[a] |
| Proliferation: PHA (10 $\mu$g/ml) | 36.01 ± 16.69 | 28.33 ± 8.12 | 89.41 ± 21.86[a] | 104.36 ± 22.29 |
| Con A (2.0 $\mu$g/ml) | 59.54 ± 15.71 | 58.61 ± 17.83 | 105.27 ± 21.42[a] | 145.86 ± 63.17[a] |
| PWM (10 $\mu$g/ml) | 30.76 ± 9.78 | 28.57 ± 11.13 | 66.41 ± 27.37 | 77.12 ± 23.18[a] |

Results are expressed as mean ± SEM.
Neutrophil number = $10^6$; Lymphocyte number = $2 \times 10^6$.
[a] $P < 0.05$.
[b] $P < 0.01$ compared to controls.
$n = 8$.

Table 5
The effects of i.c.v. administration of neuropeptide Y on neutrophil phagocytosis and mitogen stimulated lymphocyte proliferation in the rat

| | Control (medium) | NPY ($5 \times 10^{-9}$ M/5 $\mu$l) | NPY ($10^{-7}$ M/5 $\mu$l) |
|---|---|---|---|
| Phagocytosis peak height (mV) | 35.15 ± 3.16 | 30.679 ± 6.25 | 24.17 ± 5.3[a] |
| Time of onset (s) | 60.76 ± 5.73 | 65.12 ± 7.93 | 82.11 ± 8.14[a] |
| Proliferation: PHA (10 $\mu$g/ml) | 40.67 ± 10.82 | 43.88 ± 6.37 | 33.64 ± 7.56 |
| Con A (2.0 $\mu$g/ml) | 68.19 ± 14.33 | 78.72 ± 17.29 | 54.87 ± 17.54 |
| PWM (10 $\mu$g/ml) | 36.91 ± 12.31 | 38.55 ± 11.31 | 27.88 ± 5.21 |

Results are expressed as mean ± SEM.
Neutrophil number = $10^6$; Lymphocyte number = $2 \times 10^6$.
[a] $P < 0.05$ compared to the control.
$n = 8$.

lower compared with controls (i.e. neutrophils incubated with same volume of HBSS) (Table 2). JO 1784 also significantly increased the time of onset of phagocytosis (Table 2). The mitogen phytohemagglutinin, concanavalin A and pokeweed mitogen stimulated thymidine uptake was significantly reduced by sigma ligand JO 1784 in the lymphocyte proliferation test (Table 2). The suppressive effect of JO 1784 on both cellular functions was dose dependent and was significant in the concentration range of $10^{-7}$ to $10^{-5}$ M.

Neuropeptide Y also had a significant inhibitory effect on neutrophil phagocytosis causing a decreased peak height and increased time onset of phagocytosis (Table 3). Neuropeptide Y in vitro treatment also markedly reduced thymidine uptake during the lectin-stimulated lymphocyte proliferation test. The effects were significant at both doses used ($10^{-9}$ and $10^{-7}$ M) (Table 3).

### 3.3. Central effects of JO 1784 and neuropeptide Y on immune functions

Results in Table 4 show that i.c.v. administration of JO 1784 at doses of 0.5 and 5 $\mu$g/5 $\mu$l (which approximate to $5 \times 10^{-5}$ M and $10^{-4}$ M in vitro) significantly reduced the peak height of neutrophil phagocytosis and increased the onset time of phagocytosis. However, JO 1784 enhanced mitogen stimulated lymphocyte proliferation. Both of these effects were dose dependent (Table 4). I.c.v. administration of JO 1784 at doses of 0.1 $\mu$g/5 $\mu$l ($10^{-6}$ M) and 10 $\mu$g/5 $\mu$l ($2 \times 10^{-4}$ M) were also evaluated on phytohemagglutinin and concanavalin A stimulated lymphocyte proliferation; 0.01 $\mu$g/5 $\mu$l JO 1784 slightly increased lymphocyte proliferation, while 10 $\mu$g/5 $\mu$l JO 1784 slightly decreased lymphocyte proliferation. These changes did not reach statistical significance (results not shown).

The central effects of NPY infusion on neutrophil function was similar to that of JO 1784 (Table 5). Thus the phagocytic response was reduced and the time of phagocytic onset was longer in highest dose ($10^{-7}$ M) group, whereas the effect of neuropeptide Y on lymphocyte proliferation was different from that of JO 1784. Neither dose of neuropeptide Y ($10^{-9}$ and $10^{-7}$ M) affected the [$^3$H]thymidine uptake but there was a slight decrease in the lymphocyte proliferation after highest dose of neuropeptide Y used (Table 5).

### 3.4. Central administration of JO 1784 and neuropeptide Y on changes in the serum concentration of corticosterone

JO 1784 i.c.v. administration failed to change the serum concentration of corticosterone at any of the doses used (Table 6). However, both doses of neuropeptide Y significantly increased corticosterone concentration and these changes were dose dependent (Table 6).

Table 6
The effects of i.c.v. administration of JO 1784 and neuropeptide Y on the concentration of corticosterone in the rat

| | Mean | SEM |
|---|---|---|
| Control | 9.68 | 1.47 |
| JO 1784 (0.1 $\mu$g/5 $\mu$l) | 10.11 | 1.09 |
| JO 1784 (0.5 $\mu$g/5 $\mu$l) | 9.73 | 0.74 |
| JO 1784 (5.0 $\mu$g/5 $\mu$l) | 9.25 | 1.11 |
| NPY (0.5 nM) | 13.71 | 1.10[a] |
| NPY (2.0 nM) | 18.89 | 2.17[b] |

Results are expressed as mean and SEM and $\mu$g/dl.
[a] $P < 0.05$ and [b] $P < 0.01$ compared to the control.
$n = 8$.

## 4. Discussion

The results in Table 1 shows that in vitro $\sigma$ receptor ligands DTG, PCP and haloperidol significantly suppressed mitogen-stimulated lymphocyte proliferation which are similar to previous findings reported by Carr et al. (1992). It has also been shown that JO 1784 has similar immunosuppressive effects as those 'more classical' $\sigma$ receptor ligands.

In the present study, the results demonstrated for the first time that the $\sigma$ receptor ligand JO 1784 and neuropeptide Y exert similar immunosuppressive influences in vitro on some neutrophil and lymphocyte functions. These results suggest that, in vitro JO 1784 and neuropeptide Y may act in similar fashion either on $\sigma$ receptors, or

through the same intracellular pathways, to change cellular functions.

It has been reported that there are at least two types of $\sigma$ receptors, $\sigma_1$ and $\sigma_2$. $\sigma_1$ receptors are present in high concentrations in the immune and endocrine system, while $\sigma_2$ receptors are present in low concentrations (Wolfe and De Souza, 1994). So far, there is no evidence that PCP receptors occur in human peripheral lymphocytes, rat spleen and endocrine glands (Wolfe and De Souza, 1994). Carr et al. (1991, 1992)) and Garza et al. (1993) reported the existence of some $\sigma$ receptor ligand binding sites on T- and B-lymphocytes, NK cells and thymocytes. The culture of (+)-pentazocine, DTG, haloperidol and $\sigma$ receptor ligand JO 1784 in vitro with these immunocytes resulted in reductions in the NK cell activity, lymphocyte proliferation and antibody production at concentrations of $10^{-7}$–$10^{-5}$ M (Carr et al., 1991, 1992; Garza et al., 1993). These studies demonstrated that JO 1784, same as the other 'classical' $\sigma$ receptor ligands, selectively bind to $\sigma$ receptors. Other investigators have reported that the low concentration of $\sigma$ receptor ligands (below $10^{-7}$ M) appeared to act solely through $\sigma$ receptors, whereas higher concentrations ($> 10^{-5}$ M) of these ligands may also activate non-$\sigma$ receptors (Gonzalez-Alvear and Werling, 1994). For these reasons, we chose the concentrations of JO 1784 in the range of $10^{-9}$ to $10^{-5}$ M to compare the effects of neuropeptide Y on immune cellular functions.

Results in Table 2 showed that novel $\sigma$ receptor ligand JO 1784 also has suppressive effects on neutrophil phagocytosis and lymphocyte proliferation at doses $10^{-7}$ and $10^{-5}$ M but none at $10^{-9}$ M. In vitro studies have shown that JO 1784 has no affinity for PCP or any other non-$\sigma$ receptor sites in the range of concentrations used in the present study. There is also evidence that JO 1784 has selectivity for $\sigma_1$ receptors (Roman et al., 1993; Bouchard et al., 1994). The intracellular mechanisms of the action of $\sigma$ receptor ligands such as JO 1784 have been linked to the activation of G-proteins and a change in $Ca^{2+}$ uptake (Goto et al., 1983; Carr et al., 1991, 1992).

Neuropeptide Y has been reported to inhibit NK cell cytotoxicity at concentrations of $10^{-9}$ to $10^{-12}$ M (Nair et al., 1993), neutrophil chemotaxis at $10^{-7}$ to $10^{-9}$ M (Dureus et al., 1993) and neutrophil phagocytosis at $10^{-9}$ M (Hafstrom et al., 1993). Our in vitro results are consistent with these findings in that neuropeptide Y significantly suppressed the response of neutrophil phagocytosis and prolonged the onset of phagocytosis at both $10^{-9}$ and $10^{-7}$ M. This study demonstrated for the first time that treatment of neuropeptide Y in vitro significantly reduced [$^3$H]thymidine uptake during lymphocyte proliferation.

There are two types of neuropeptide Y receptors, neuropeptide Y $Y_1$ and neuropeptide Y $Y_2$, which are widely distributed in the mammalian brain, sympathetic nervous system, immune organs and endocrine glands (Romano et al., 1991; Dumont et al., 1992; Wahlestedt and Reis, 1993). While the difference in distribution of these two receptors has been studied in the brain, there is no available information regarding the distribution of these receptors in the immune system. The intracellular mechanisms of neuropeptide Y in vitro in modulating lymphocyte and neutrophil functions is also unclear. It has been reported that neuropeptide Y raises the intracellular $Ca^{2+}$ concentrations in many cell types including neutrophils (Motulsky and Michel, 1988; Mihara et al., 1989; Aakerlund et al., 1990; Hafstrom et al., 1993). In addition, neuropeptide Y acts upon its membrane receptors that are G-protein linked (Unden and Bartfai, 1984; Wahlestedt et al., 1990), which is similar to the cellular mechanism of $\sigma$ receptor ligands.

Results from investigations in the CNS have suggested that neuropeptide Y can act as direct potent competitors of $\sigma$ receptors labelled using (+)[$^3$H]SKF10047 (Roman et al., 1989, 1990). In addition, it has been found that both neuropeptide Y and JO 1784 reduced intestinal ion transport; effects which were attenuated by $\sigma$ antagonist haloperidol (Monnet et al., 1990). Therefore, the action of neuropeptide Y has been proposed as an endogenous ligand for the $\sigma$ receptor (Roman et al., 1989; Riviere et al., 1990). However, to date no studies have investigated the possible relationship between these two agents on the immune system. The evidence from the present study may indicate that JO 1784 and neuropeptide Y trigger the same intracellular pathways in modulating the activity of some types of immune cells.

The precise relationship between neuropeptide Y receptors and $\sigma$ receptors is still uncertain. Su (1993) reported that $\sigma$ receptors differ from both neuropeptide Y $Y_1$ and neuropeptide Y $Y_2$ receptors in their distribution and biochemical characteristics. Furthermore, while there is evidence that neuropeptide Y and the related peptide YY alter the functional activity of $\sigma$ receptors in mouse brain, there is no evidence that these peptides have any affinity for $\sigma$ receptors (Tam and Mitchell, 1991; Quirion et al., 1991; Roman et al., 1993). Clearly further research is required to determine the precise relationship between neuropeptide Y and $\sigma$ receptors. For example, some antagonists for $\sigma$ binding sites should be investigated together with neuropeptide Y.

Following the central administration of JO 1784 or neuropeptide Y, the peak height of neutrophil phagocytosis was significantly reduced and the time of onset of phagocytosis was increased. However, the influences of JO 1784 and neuropeptide Y on lymphocyte proliferation and the serum concentration of corticosterone clearly differ. Both JO 1784 and neuropeptide Y stimulate noradrenaline and dopamine release from the hippocampus, hypothalamus and other brain regions by activating NMDA receptors (Heilig et al., 1990; Monnet et al., 1992a,b). However, the action of most $\sigma$ receptor ligands on the brain and pituitary gland appears to be indirectly mediated by dopamine, opiate or PCP receptors (Wolfe et al., 1989; Zhang et al., 1993; Barg et al., 1994). By contrast, there is evidence that neuropeptide Y modulates corticotropin-releasing factor

(CRF) and adrenoceptors in the CNS and the sympathetic nervous system that innervates the lymphoid organs (Wahlestedt et al., 1989; Heilig et al., 1990; Romano et al., 1991; Schalling et al., 1991). Furthermore, Nagain et al. (1995) have shown that i.c.v. injections of neuropeptide Y inhibited pancreatic secretion by acting on $\alpha_2$-adrenoceptors, an effect that $\sigma$ receptor ligands lack. Thus, whereas $\sigma$ receptor ligands may exert their modulatory effects on the immune and endocrine system directly through actions on the pituitary and target organs (Wolfe and De Souza, 1993), NPY may produce its immunomodulatory effects indirectly by activating the sympathetic system which innervates the lymphoid organs (Felten et al., 1992; Irwin et al., 1992). These difference between the sites of action of $\sigma$ receptor ligands and neuropeptide Y may be responsible for their different actions on immune and endocrine system which have been shown in the present study.

It has been shown that i.c.v. administration of most $\sigma$ receptor ligands significantly elevate corticosterone by activating serotonin and dopamine receptors in the CNS and/or $\sigma$ receptors in the pituitary gland (Gudelsky and Nash, 1992; Karbon et al., 1993; Wolfe and De Souza, 1994). In the present study, the highly specific $\sigma_1$ receptor ligand JO 1784 did not change the corticosterone concentration. This result agrees with our previous finding in which chronic treatment with JO 1784 also failed to change corticosterone concentrations in the rat (Song and Leonard, 1997). One explanation for this is that non-selective $\sigma$/PCP receptors agonists stimulate the pituitary to release adrenocorticotropin hormone (ACTH) whereas selective $\sigma$ receptor ligands (such as JO 1784) have little effect on the release of ACTH (Wolfe and De Souza, 1994). In support of this view, we have recently shown that JO 1784 failed to reverse a CRF-induced increase in serum corticosterone or CRF induced changes in lymphocyte and neutrophil numbers (Song and Leonard, 1997).

Several studies have demonstrated that i.c.v. administration of neuropeptide Y (at doses higher than $10^{-9}$ M) stimulates the secretion of CRF from the hypothalamus thereby increasing the concentrations of ACTH and corticosterone; as a consequence the raised serum corticosterone suppresses some aspects of cellular immunity (Irwin et al., 1991; Song et al., 1994, 1995). We have previously found that incubation of corticosterone (25 to 100 $\mu$g/dl) with neutrophils significantly reduces the peak height of phagocytosis and prolongs the onset time of the phagocytic response (data not published). Thus the changes caused by i.c.v. neuropeptide Y administration found in the present study are consistent with the view that some of the effects of this peptide on cellular immunity are due to the rise in the serum glucocorticoid concentration.

In conclusion, we have demonstrated that both JO 1784 and neuropeptide Y in vitro exert an immunosuppressive influence on neutrophil phagocytosis and mitogen stimulated lymphocyte proliferation. These effects may be elicited by the activation of G-proteins, change of $Ca^{2+}$ uptake or by a functional increase in $\sigma$ receptors. The central administration of JO 1784 significantly reduced the activity of neutrophil phagocytosis, but enhanced lymphocyte proliferation without changing the concentration of corticosterone. By contrast, neuropeptide Y infusion also decreased neutrophil responsiveness but significantly raised the serum corticosterone concentration. These results indicate that different mechanisms related to an increase in amine neurotransmitters and their receptors. Changes in the HPA axis and in the activity of $\sigma$ receptors may be involved in the central modulation following JO 1784 and neuropeptide Y administration. It therefore seems unlikely that specific $\sigma$ receptor ligands such as JO 1784, and neuropeptide Y cause changes in the immune system by a common action on $\sigma$ receptors.

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