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Modulation of Immune Cell Function Following Fluoxetine Administration in Rats

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PELLEGRINO, T. C. AND B. M. BAYER. *Modulation of immune cell function following fluoxetine administration in rats.* PHARMACOL BIOCHEM BEHAV **59**(1) 151–157, 1998.—Fluoxetine (FLX) and other specific serotonin uptake inhibitors (SSRIs) have become the drugs of choice for treating depression. However, only a limited number of studies have addressed the effects of FLX on immune cell function. Our lab has measured the effects of both acute and chronic FLX administration on two functions of cell-mediated immunity, mitogen-induced lymphocyte proliferation (MILP) and natural killer cell cytolytic activity (NKCA). In this article we report that acute FLX administration (10 mg/kg) resulted in a dose- and time-dependent decrease in MILP and NKCA. MILP was more sensitive than NKCA to FLX, requiring lower doses for inhibition; however, the effects were more transient. Following chronic FLX administration, these effects were no longer observed, suggesting that an apparent tolerance to these particular measures of cell-mediated immunity had developed. Finally, a single microinjection of FLX directly into the lateral ventricle produced similar suppressive effects on MILP and NKCA, suggesting that the immunomodulatory mechanism may have a central component. © 1998 Elsevier Science Inc.

Fluoxetine Serotonin Cell-mediated immunity Lymphocytes T-cells NK cells
Specific serotonin reuptake inhibitors Proliferation Cytolytic activity Chronic Acute Central

PATIENTS suffering from depression have been shown to have a decrease in certain cell-mediated immune cell functions (10,14,25–27,30,33,46,47). The most widely reported immune cell deficiencies have been decreases in mitogen-induced lymphocyte proliferation (MILP) (10,46,47) and natural killer cell cytolytic activity (NKCA) (10,14,25–27,30,33). Consistent with an overall immunosuppressive state, an increased incidence of cancer has also been observed with depression (22,39), especially in those depressed patients exposed to environmental carcinogens (32).

Fluoxetine (FLX) and other specific serotonin reuptake inhibitors (SSRIs) have become the drugs of choice for treating depression, with relatively few side effects (18,23,29,40,43,50,52). However, only a limited number of studies have addressed the effects of FLX on immune cell function. Patients receiving FLX treatment have been reported to experience reactivation of herpes simplex infections (42) and develop cutaneous pseudolymphoma lesions (13), both of which resolve with discontinuation of FLX therapy. These observations indicate that cell-mediated immunity may be disrupted in these patients as a result of drug treatment. In studies carried out in rodents, both chronic and acute administration of FLX, have been shown to have effects consistent with a compromised immune system. For example, chronic FLX administration has been shown to increase the growth rate of transfected tumors in mice and chemically induced tumors in rats (8). In addition, acute FLX has been shown to decrease immune cell infiltration and inflammation in rats (6,7).

Although MILP and NKCA are the two most commonly measured immune cell parameters in patients with depression, very little information is available on the effects of SSRIs on these two immune parameters. Therefore, we investigated whether acute FLX administration resulted in changes in these two independent measures of cell-mediated immunity. In these studies, both time- and dose-dependency of the immunomodulatory effects of FLX were characterized. Because the therapeutic use of FLX is predominantly over extensive periods of time, the effect of chronic FLX treatment was also examined. In addition to peripheral administration, FLX was also microinjected unilaterally into the lateral ventricle, to determine whether central serotonergic pathways may be involved in the modulation of peripheral immune cell function.

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METHOD

Animals

Virus-free male Sprague–Dawley rats (225–325 g, Taconic) were housed three per cage, with microisolator tops. Animals were housed for 1 week to acclimate after receipt, and were maintained on a 12 L:12 D cycle with food and water ad lib. For chronic administration studies, rats were weighed every other day prior to injection.

Drugs and Administration

Fluoxetine HCl (Prozac®, Eli Lilly and Company, Indianapolis, IN) was dissolved in warm sterile saline. For acute systemic administration, FLX or sterile saline (1 ml/kg) were injected intraperitoneally (IP) 2 h prior to sacrifice, unless otherwise noted. For chronic treatment, FLX or sterile saline were administered IP once daily for 2 weeks. Fluoxetine was administered at a dose of 10 mg/kg, unless otherwise stated.

For central administration, drug solutions were administered, in freely moving rats, into the lateral ventricle via an injection cannula inserted through a permanently implanted guide cannula. FLX (10 μg/μl) or sterile saline were administered, via microinfusion pump, in a total volume of 5 μl over 30 s (50 μg/rat).

Stereotaxic Cannulation

Upon receipt, animals were allowed to acclimate 1 week prior to surgery. Under Equithesin anesthesia, guide cannulae were stereotaxically implanted unilaterally into the lateral ventricle as previously described in our laboratory (21). Paxinos and Watson was referenced from bregma for lateral ventricle coordinates (AP = −0.9, DV = −3.4, ML = −1.5) (37). Gentamicin (8 mg) was administered subcutaneously following surgery, and animals were allowed to recover for 1 week prior to use in an experiment.

Mitogen-Induced Lymphocyte Proliferation

Rats were sacrificed by decapitation and trunk blood was collected into heparinized tubes. Whole blood was diluted 1:5 (WB1:5) and 1:10 (WB1:10) with RPMI-1640 media 1% fetal calf serum (FCS), and 100 μl of each suspension was added in triplicate to 96-well flat-bottomed microtiter plates containing 100 μl of concanavalin A (Con A) solution (WB1:5 = 0, 0.2, 0.4, 0.6 μg/culture and WB1:10 = 0, 0.1, 0.2, 0.4 μg/culture). At these concentrations of mitogen, submaximal and maximal proliferation responses were obtained. Cultures were incubated for 72 h at 37°C with 8% CO_2 and then pulsed with 0.5 μCi/well of [^{3}H]CH_3-thymidine (6.6–6.7 Ci/mmol, NEN, Wilmington, DE) for 24 h. Cells were lysed by deionized water using a 96-well cell harvester (Brandel, Gaithersburg, MD) and labeled DNA was collected onto glass fiber filtermats. Radioactivity was quantitated by liquid scintillation spectrophotometry (Betaplate, L.K.B. Pharmacia).

NK Cell Cytolytic Activity (Chromium Release Assay)

Spleens were removed immediately following sacrifice, and spleen cells (effector cells) were collected by teasing spleens gently in RPMI-1640 media with 1% FCS. The cells were pelleted by centrifugation (200 × g), washed once with RPMI-1640 media (1% FCS), and resuspended in RPMI-1640 media (10% FCS) at a final concentration of 10×10^6 cells/ml. For the NK assay, the cell suspension was serially diluted to a concentration of 5, 2.5, and 1.25×10^6 cells/ml and 100 μl of each suspension was added in triplicate to a 96-well round-bottom microtiter plate. YAC-1 cells (target cells), a mouse lymphoma cell line, were labeled by incubating 0.3 ml of cell suspension (10×10^6 cells/ml) in serum-free RPMI media for 1 h with 100 μCi Na_2-$^{51}CrO_4$ (435–470 mCi/mg, NEN, Wilmington, DE). Labeled cells were then washed and resuspended in RPMI (10% FCS) to a final concentration of 1×10^5 cells/ml and added (100 μl) to the splenocyte cultures. For determination of spontaneous release (SR), 100 μl of media alone were added to six wells containing 100 μl of labeled YAC-1 cells. Total activity (TA) was determined by directly counting 100 μl of the labeled YAC-1 cell suspension. After a 4 h incubation at 37°C with 8% CO_2, a 100 μl aliquot of the supernatant was collected from each well and counted by liquid scintillation spectrophotometry. Percent cytolytic activity was determined by dividing the cpm for the sample by the TA after correcting each for spontaneous release.

Statistics

An analysis of variance test for multiple comparisons, with Newman–Keuls post hoc analysis was performed to determine statistical significance for these studies. For lymphocyte proliferation, the median of each triplicate was selected and the maximum response in each dose–response curve was determined for graphing and statistics. For NK cell activity, the median of each triplicate was selected and the mean of each effector:target ratio was determined. When data are represented as a full Con A dose response or all effector:target ratios, significance was determined via two-way analysis of variance with replications and Newman–Keuls post hoc analysis.

RESULTS

The acute effects of FLX were first determined on whole-blood MILP, using the T-cell specific mitogen, Con A, and splenic NKCA 2 h postinjection. A dose of 0.1 mg/kg of FLX had no significant effect on MILP at this time (Fig. 1A). However, FLX at doses of 1 and 10 mg/kg was found to significantly suppress MILP by 50 and 56%, respectively (Fig. 1A), $F(3, 23) = 4.76$, $p < 0.01$. Similar to the effects on blood MILP, splenic NKCA was also inhibited, but only with the higher dose of FLX (10 mg/kg) (Fig. 1B), $F(3, 29) = 4.54$, $p < 0.01$. Maximal inhibition was 34% at the 50:1 effector:target cell ratio (Fig. 1B) and a similar magnitude of inhibition was observed at the 12.5:1 and 25:1 effector:target cell ratios (data not shown).

The acute suppressive effects of FLX on MILP and NKCA were found to be transient with slight differences in their recovery times. Maximal inhibition (55%) of MILP was observed within 2 h following FLX (10 mg/kg) (Fig. 2A), $F(4, 31) = 4.02$, $p < 0.01$. Responses remain suppressed at 4 h, $F(4, 31) = 3.01$, $p < 0.05$; however, by 18 h the MILP response was no longer suppressed. In contrast to MILP, the inhibitory effects of acute FLX administration on NKCA appeared to be more prolonged (Fig. 2B). Significant suppression (50%) was observed by 2 h, and similar magnitude of suppression (55%) remained at 4 h, $F(3, 26) = 4.64$, $p < 0.01$. Unlike MILP, NKCA activity was still significantly lower than control at 18 h following FLX treatment, $F(3, 26) = 4.64$, $p < 0.01$.

To determine whether the immune effects seen acutely with FLX persisted after chronic administration, FLX (10 mg/kg, IP) was administered once daily for 2 weeks. As expected, chronic administration of FLX had a profound anorectic effect on the animals predominantly during the first week of treatment (Fig. 3) [treatment, $F(7, 112) = 3.12$, $p < 0.01$]. Al-

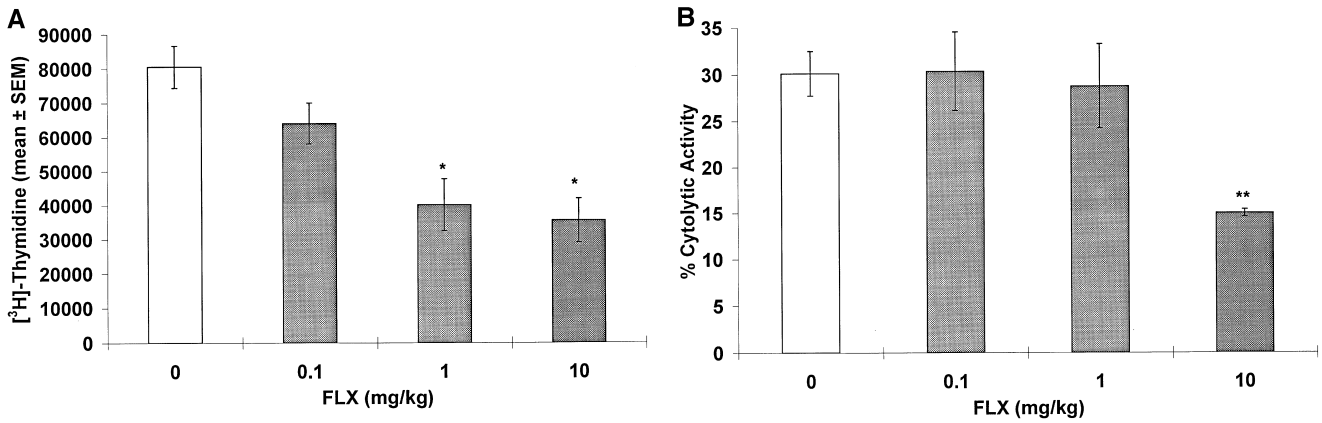

FIG. 1. Inhibition of blood lymphocyte proliferation and NK cell cytolytic activity 2 h after fluoxetine administration. Animals were injected intraperitoneally with saline (1 ml/kg), or fluoxetine (0.1, 1, 10 mg/kg). (A) Two hours after drug administration, blood was collected into heparinized tubes. Lymphocyte proliferation in WB1:10 to Con A (0.1, 0.2, and 0.4 μg/well) was determined as described in the Method section. Maximum proliferative responses were chosen for graphing and statistics. Results are representative of two independent experiments. Data are expressed as the mean ± SEM [^{3}H]CH_3-thymidine cpm/culture of saline and fluoxetine treated (0.1, 1, or 10 mg/kg) animals per group (n = 7). (B) Two hours after drug administration intraperitoneally with saline (1 ml/kg) or fluoxetine (0.1, 1, or 10 mg/kg), splenic NK cells were collected and cytolytic activity was measured as described in the Method section. Maximal results (50:1 effector:target ratio) are shown, and are expressed as the mean ± SEM percent specific lysis of saline and fluoxetine-treated animals per group (n = 6–14). *Denotes significant difference from saline-treated group, as determined by ANOVA, *$p < 0.01$.

though animals began to gain weight during the second week, the average body weight was 17% lower than saline-injected controls at the end of the treatment, $F(3, 28) = 4.57$, $p < 0.01$. Nevertheless, no significant effect was observed on either MILP or NKCA 2 h following the last dose of FLX in chronically treated animals (Fig. 4A and B). As a positive control, a third group of saline-injected animals were acutely administered FLX (10 mg/kg) and sacrificed 2 h later along with the chronically treated animals. As previously observed (Figs. 1 and 2), there was a significant decrease in both MILP, $F(2, 18) = 6.01$, $p < 0.01$, and NKCA, $F(7, 56) = 3.29$, $p < 0.01$; treatment, $F(3, 56) = 4.31$, $p < 0.01$, in the group treated acutely with FLX (Fig. 4A and B).

To determine whether the effects of FLX on MILP and NKCA may be modulated by central serotonergic modulation, FLX was administered unilaterally into the lateral ven-

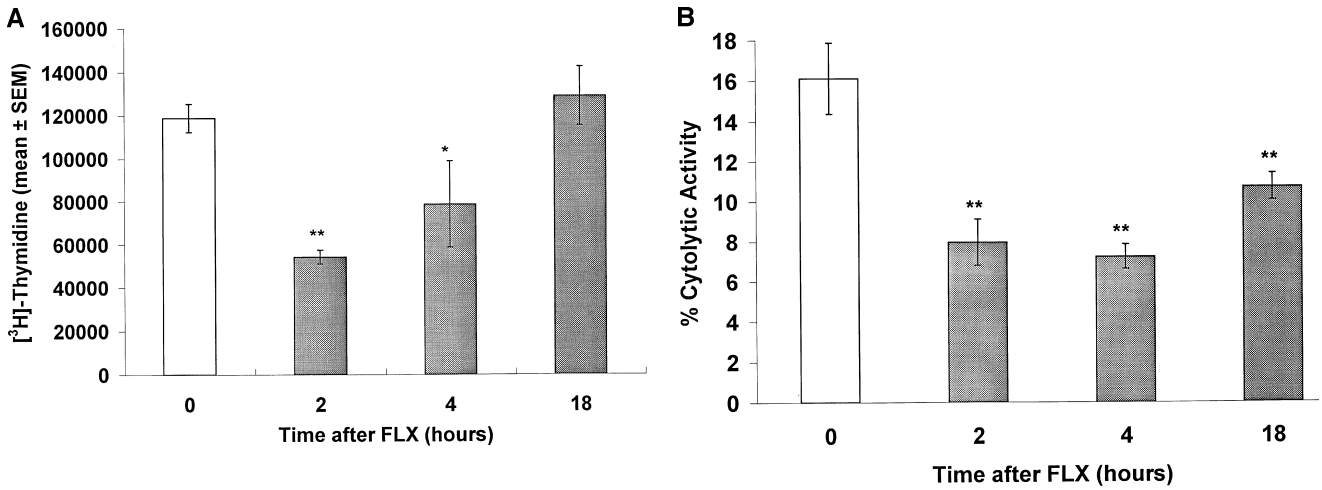

FIG. 2. Time-dependent inhibition of blood lymphocyte proliferation and NK cell cytolytic activity following fluoxetine administration. (A) Animals were injected intraperitoneally with saline (1 ml/kg), or fluoxetine (10 mg/kg). At the indicated time after drug administration, blood was collected into heparinized tubes. Lymphocyte proliferation in WB1:5 to Con A (0.2, 0.4, 0.6 μg/well) was determined as described in the Method section. Maximum proliferative responses were chosen for graphing and statistics. Results are representative of two independent experiments. Data are expressed as the mean ± SEM [^{3}H]CH_3-thymidine cpm/culture of saline and fluoxetine treated (2, 4, or 18 h) of seven to nine animals per group. (B) At the indicated time after drug administration, as described in (A), splenic NK cells were collected and cytolytic activity was measured as described in the Method section. Maximal results (50:1 effector:target ratio) are shown, and are expressed as the mean ± SEM percent specific lysis of saline and fluoxetine-treated animals animals per group (n = 7–9). Denotes significant difference from saline-treated group, as determined by ANOVA, *$p < 0.05$, **$p < 0.01$.

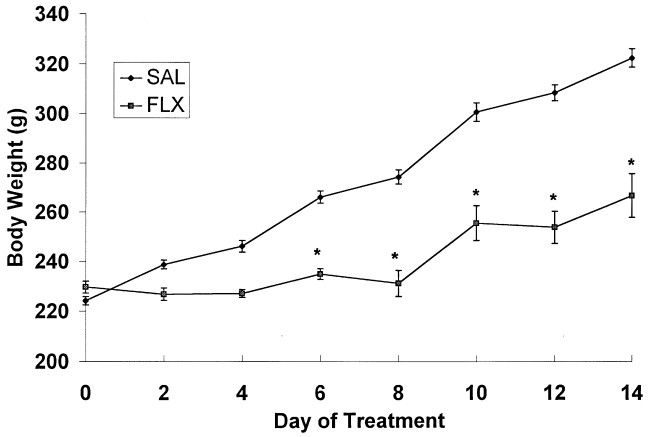

FIG. 3. Effect of chronic fluoxetine administration on body weight. Animals were injected intraperitoneally once a day with 10 mg/kg of fluoxetine or saline. The body weight was measured every other day, and results were recorded for each animal. Data were expressed as mean ± SEM for each group (saline, $n = 15$ and fluoxetine, $n = 8$). The body weights from the saline and the acute fluoxetine groups were combined, because they were both chronically treated with saline. Denotes significant difference from saline-treated group, as determined by ANOVA, $^*p < 0.01$.

tricle and the animals were sacrificed 2 h later. As observed with peripheral administration, central microinjection of FLX (50 μg) resulted in a significant decrease in both MILP, $F(7, 44) = 3.29$, $p < 0.01$; treatment, $F(3, 40) = 8.3$, $p < 0.01$, and NKCA, $F(7, 40) = 2.34$, $p < 0.05$, $F(7, 40) = 3.29$, $p < 0.01$; treatment, $F(3, 40) = 4.31$, $p < 0.01$, within 2 h (Fig. 5A and B). The magnitude of the inhibition with both immune parameters was similar to that seen following peripheral administration.

DISCUSSION

In this report we have shown that acute fluoxetine administration results in a dose- and time-dependent decrease in mitogen-induced lymphocyte proliferation and natural killer cell cytolytic activity. There were some differences between the drug sensitivity and duration of action in the MILP and NKCA responses to acute FLX treatment. NKCA was not as sensitive as MILP to the suppressive effects of FLX, because a higher dose of FLX (10 mg/kg) was required to significantly decrease NK cell function compared to MILP (1 mg/kg). However, the effects of FLX on NKCA were more prolonged than the effects on MILP. NKCA was still significantly suppressed at 18 h, whereas MILP responses had returned to control values at this time. Following chronic FLX administration, the suppression of both the immune parameters was no longer observed, suggesting that an apparent tolerance had developed. Finally, a single microinjection of FLX unilaterally into the lateral ventricle produced similar suppressive effects, suggesting that central modulation of serotonergic pathways may be involved in modulating NKCA and MILP.

There are several factors that alone, or in combination, could be contributing to the transient effects seen following acute FLX administration. First, the half-life of FLX has been reported to be between 4–7 h in rats (9). Therefore, the lack of significant effects by 18 h may simply be due to elimination of FLX from the system. Second, following acute exposure to FLX, a desensitization or downregulation of serotonergic receptors may contribute to the transient response. Acute stimulation of serotonin receptors has been reported to lead to desensitization within 2 h in vitro (28) and within 24 h in vivo (44). Third, single doses of FLX have been shown to decrease serotonergic neurotransmission due to activation of presynaptic autoreceptors (1). Initially, when FLX binds to the serotonin uptake pump, there is an increase in serotonin in the synapse. However, elevation of synaptic serotonin will activate the presynaptic autoreceptors and eventually decrease seroto-

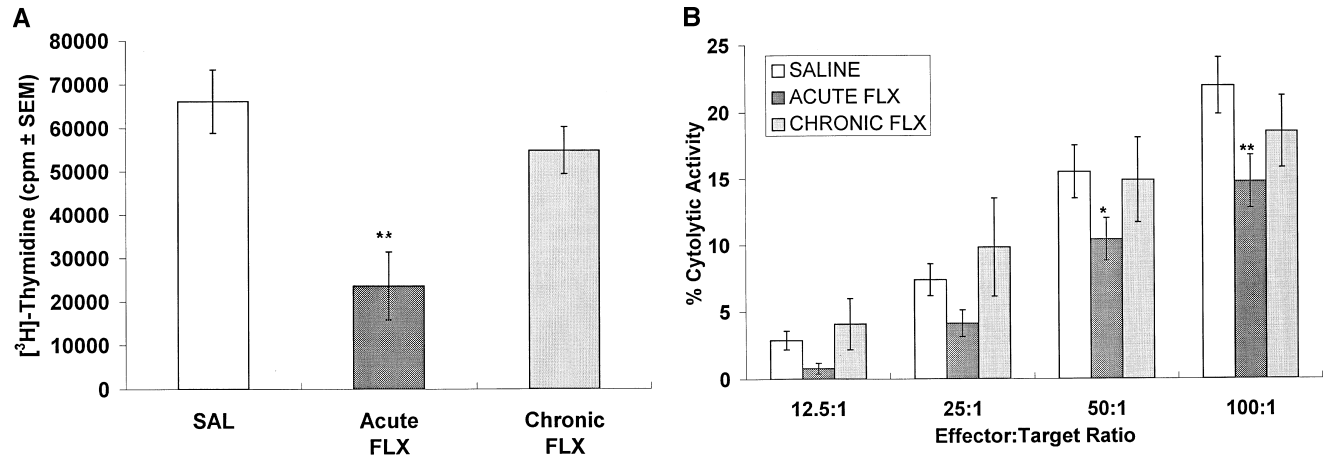

FIG. 4. Inhibition of MILP and NKCA after chronic administration of fluoxetine systemically. Animals were injected intraperitoneally once daily for 14 days with saline (1 ml/kg) or fluoxetine (10 mg/kg). On day 14 the saline-treated animals were separated into two groups: one receiving another dose of saline, and one receiving an acute dose of fluoxetine (10 mg/kg). The chronic fluoxetine group received a final fluoxetine injection. (A) Two hours after the final drug administration, blood was collected as described in the legend for FIG. 2A. Results are representative of two independent experiments. Data are expressed as the mean ± SEM $[^3H]CH_3$-thymidine cpm/culture of saline, acute fluoxetine, and chronic fluoxetine-treated animals per group ($n = 7$–9). (B) Two hours after the final drug administration, as described in A, splenic NK cells were collected and cytolytic activity was measured as described in the Method section. The full effector:target ratio curve is shown, and results are expressed as the mean ± SEM percent specific lysis of saline, acute fluoxetine and chronic fluoxetine treated animals per group ($n = 6$–8). Denotes significant difference from saline-treated group, as determined by ANOVA, $^*p < 0.05$, $^{**}p < 0.01$.

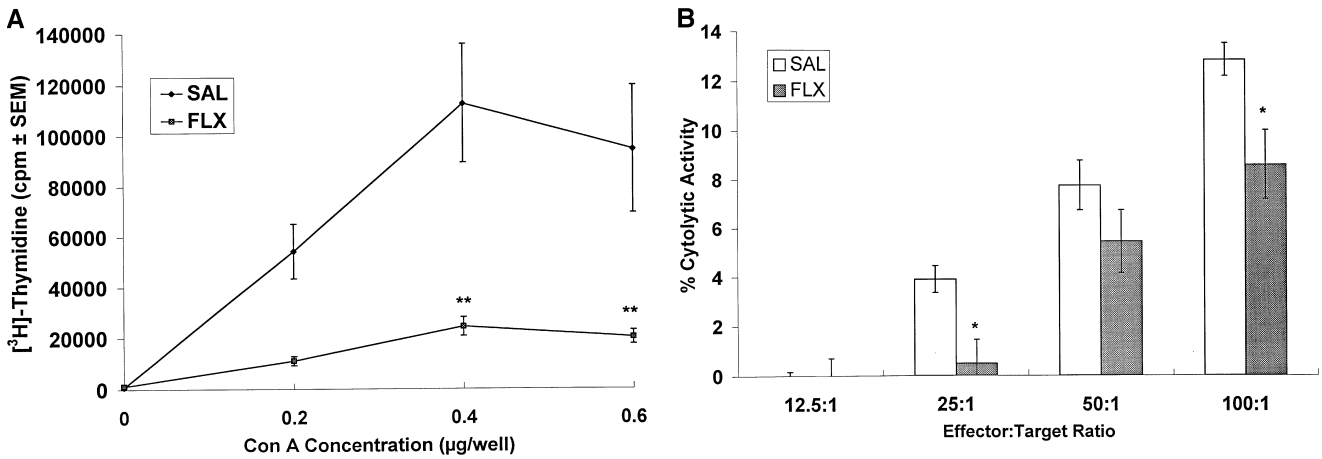

FIG. 5. Inhibition of MILP and NKCA 2 h after central administration of fluoxetine into the lateral ventricle. Animals were injected centrally with saline (5 µl) or fluoxetine (50 µg). (A) Two hours after drug administration, blood was collected as described in the legend for Fig. 2A. The full Con A dose–response is shown, and data are expressed as the mean ± SEM [^{3}H]CH_3-thymidine cpm/culture of saline and fluoxetine-treated animals per group (n = 9–12). (B) Two hours after drug administration, as described in legend of Fig. 1B, splenic NK cells were collected and cytolytic activity was measured as described in the Method section. The full effector:target ratio curve is shown and results are expressed as the mean ± SEM percent specific lysis of saline and fluoxetine-treated animals per group (n = 12–13). Denotes significant difference from saline-treated group, as determined by ANOVA, *$p < 0.05$, **$p < 0.01$.

nin release (1). This transient increase in serotonergic neurotransmission varies in different regions of the brain. Following FLX (10–20 mg/kg) administration, 5-HT levels are significantly elevated for less than 2 h in the frontal cortex, whereas they are elevated for greater than 3 h in the raphe nucleus and the ventral hippocampus (34). If the immune cell response is being regulated by central mechanisms, the alteration of the activity of neurons in specific areas of the brain may be important in modulating the immune response.

The effects of FLX on cell-mediated immune function were no longer observed after chronic FLX administration. Therefore, continued administration of FLX appeared to produce the development of a compensatory mechanism, which overcomes the effects of FLX on MILP and NKCA. As discussed above, this compensation could be related to the downregulation of receptors involved in the immunomodulatory pathway. Some serotonin receptor subtypes have been shown to downregulate following chronic treatment with FLX and other serotonergic agents (11,19,31,45,48). A similar development of an apparent tolerance to MILP has been found in our laboratory following chronic morphine and cocaine administration (3,4). However, the lack of immune cell suppression following chronic treatment does not necessarily indicate that the immune status of these animals is normal. It is possible that these compensatory mechanisms may not be adequate under situations such as stress, infection, or malignancy, thus rendering the animal more susceptible to such immune challenges (3). Therefore, further studies are necessary to further evaluate the immune response of these animals when challenged following chronic fluoxetine administration to determine if these changes could be involved in the effects of chronic FLX on immune deficiencies that have been reported, such as increases in tumor growth (8).

In this regard, it is important to make the distinction that the observations reported here were made in normal animals. Depression, for which fluoxetine therapy is typically initiated, has itself been associated with many endocrine changes similar to those observed in a chronic stress state (12,15–17,24,38,51). In addition, humans with depression are well known to have accompanying immune dysfunction (10,14,25–27,30,35,36,46,47). Some studies have shown that changes in NK cell number and levels of certain cytokines, such as IL-6, seen in depressed patients are normalized following chronic SSRI treatment (41,49). The effect of FLX on MILP and NKCA in a disease state, such as depression, in which the immune competence is already compromised has yet to be determined.

Because direct administration of FLX into the lateral ventricle decreases MILP and NKCA in a similar manner to systemically administered FLX, the effects of FLX appear to be mediated, at least in part, by modulation of central serotonergic neurotransmission. However, direct effects of FLX on lymphocyte responses cannot yet be ruled out, because we and others have found that FLX and other serotonergic agents have suppressive effects in vitro (2,5,20,36). However, the concentration of FLX required to significantly decrease lymphocyte proliferation was greater than 100 µM (5). This is approximately 20 times greater than the peak concentration (5 µM) following an intravenous dose of FLX (10 mg/kg) in vivo (9). Therefore, at the doses used in the present study it is more likely that the primary mechanism of immunomodulation following systemic FLX is mediated by an indirect mechanism, such as an alteration in central serotonergic neurotransmission.

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