Immunology 1985 **54** 505

Influence of serotonin on the immune response

J. C. JACKSON,* R. J. CROSS,* R. F. WALKER,† W. R. MARKESBERY,†‡§ W. H. BROOKS¶ & T. L. ROSZMAN*

**Departments of Medical Microbiology and Immunology, ‡Pathology, §Neurology, †The Sanders-Brown Research Center on Aging, and ¶the Division of Neurosurgery, University of Kentucky Medical Center, Lexington, Kentucky, U.S.A.*

Accepted for publication 29 October 1984

Summary. The present study investigates the influence of pharmacological agents known to regulate biosynthesis of the neurotransmitter, serotonin (5-hydroxytryptamine, 5-HT) on the primary antibody response to sheep red blood cells (SRBC) in the CBA mouse. Systemic administration of 5-HT (4–100 mg/kg) or its precursor, 5-hydroxytryptophan (5-HTP, 50–400 mg/kg), 30–60 min before immunization resulted in dose-dependent suppression of both the IgM and IgG plaque-forming cell (PFC) response to SRBC. Conversely, para-chlorophenylalanine (PCPA, 250 mg/kg), which inhibits the rate-limiting enzyme (tryptophan hydroxylase) in 5-HT biosynthesis, markedly enhanced IgM and IgG antibody production when injected 48 hr prior to antigen. Effects of these drugs on immune processes appeared independent of observed changes in plasma corticosterone levels. Further, immune function was preserved following selective depletion of brain serotonin through intracisternal injection of the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) in mice pretreated with desmethylimipramine (DMI). Thus, immunomodulation by serotonin appears to be mediated via peripheral mechanism(s).

Correspondence: Dr J. C. Jackson, Dept. Medical Microbiology and Immunology, The Albert B. Chandler Medical Center, University of Kentucky, Lexington, KY 40536, U.S.A.

INTRODUCTION

The immune system generally is viewed as being autoregulated by a variety of mechanisms which include subsets of lymphocytes, antibodies and cytokines. It is becoming increasingly evident, however, that extrinsic factors are capable of influencing immune responsiveness. Principal among these is the central nervous system (CNS), as data continues to accrue indicating a link between the brain and the immune system (Besedovsky, Del Ray & Sorkin, 1983a). Ablation of specific nuclei of the hypothalamus results in broad suppression of lymphocyte reactivity (Cross *et al.*, 1980; Cross *et al.*, 1984; Keller *et al.*, 1980; Luparello, Stein & Park, 1964; Roszman *et al.*, 1982; Tyrey & Nalbandov, 1972). Electrophysiological (Besedovsky *et al.*, 1977) and neurochemical (Besedovsky *et al.*, 1983b) studies show that changes in neuronal activity and transmitter synthesis occur at the peak of humoral responsiveness. Thus, it is evident that an exchange of information between the brain and the mediators of immunity occurs during the generation of an immune response.

The findings that receptors for a variety of hormones (Abraham & Buga, 1976; Bathena *et al.*, 1981; Csaba, Sudan & Dobozy, 1977; Goodwin *et al.*, 1979; Hazum, Chang & Cuatrecasas, 1979; Helderman & Strom, 1978; Homo *et al.*, 1980; Lesniak *et al.*, 1977; Ogden and Hill, 1980; Smith *et al.*, 1977) and neurotransmitters (Eliseeva & Stefanovich, 1982; Flem-

inger, Jenner & Marsden, 1982; Le Fur *et al.*, 1981; Loveland, Jannott & McKenzie, 1981; Maslinski, Grabczewska & Ryzewski, 1980; Pochet *et al.*, 1979; Richman *et al.*, 1981; Roszman *et al.*, 1984; Vetoshkin, Fomenko & Zozulia, 1982; Watanabe, Lai & Yoshida, 1981) are present on the surface membrane of the lymphocyte and/or monocyte suggest that these messengers are the link between the CNS and the immune system and, hence, are capable of influencing immunity. Although the effects of hormones on lymphocyte function are well known, the role of neurotransmitters in regulating immunity is less documented. Eremina & Devoino (1973) have demonstrated that the humoral response to bovine serum albumin is enhanced following electrolytic lesioning of serotonergic neurons in the midbrain raphe nucleus. Hence, it was suggested that serotonin plays an inhibitory role in the regulation of the immune response.

The present investigation confirms and extends this hypothesis by demonstrating that the *in vivo* primary antibody response to sheep red blood cells (SRBC) is influenced by alterations in serotonin levels. This modulation of the SRBC antibody response is not related to changes in circulating corticosterone. Specific depletion of serotonin in the brain does not alter the antibody response, suggesting that modulation by serotonin occurs at the level of the lymphocyte and/or macrophage and not through alterations in central, neural serotonergic pathways.

MATERIALS AND METHODS

Animals

CBA mice, 8–12 weeks of age (Jackson Laboratories, Bar Harbor, MA) were housed in Bioclean laminer flow cabinets (Class 100 environment Bioclean, Dwyer Instruments, Inc., Michigan City, IN) and maintained on standard diet, with free access to water.

Drug treatments

Serotonin and 5-hydroxytryptophan (5HTP, Sigma Chemical Company, St Louis, MO) were administered subcutaneously, 30 min prior to immunization with antigen. Parachlorophenylalanine (PCPA, Sigma) was injected subcutaneously 48 hr before antigen. Control animals received 0·2 ml acidified saline subcutaneously, in lieu of drug.

In order to investigate the effect of central destruction of serotonergic neurons, the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT, Sigma) was injected intracisternally (100 μg in 10 μl saline) 48 hr before antigen. Although 5,7-DHT efficiently destroys serotonin containing neurons, it does produce significant lesions in catecholaminergic neurons (Björklund, Baumgarten & Nobin, 1974). An additional group of animals was therefore pretreated 1 and 4 hr before injection of 5,7-DHT, with 25 mg/kg desmethylimipramine (DMI, provided by the Merrell Dow Research Center, Merrell Dow Pharmaceutics, Inc., Cincinnati, OH) intraperitoneally, to improve the selectivity of the 5,7-DHT action for serotonergic neurons (Björklund, Baumgarten & Rensch, 1975) by blocking uptake into catecholamine-containing neurons.

Quantitation of serotonin

Serotonin concentrations were determined on total brain homogenates from animals treated with 5,7-DHT, employing a radioenzymatic technique (Saavedra, Braunstein & Axelrod, 1973). Mice were killed 4 or 6 days after inoculation with either 5,7-DHT or saline, the brains removed, frozen on dry ice, and stored at $-70°$ prior to assay of serotonin.

Quantitation of corticosterone

In order to determine the effect of drug treatments on plasma corticosterone levels, mice were inoculated subcutaneously with saline, PCPA (250 mg/kg), 5HTP (100 mg/kg), or serotonin (0·4 mg/kg). At 1, 3, 6, 12 or 24 hr after injection, the mice were bled and the plasma retained for quantitation of corticosterone by radioimmunoassay, using a commercial kit (Radioassay System Laboratories Inc., Carson, CA).

Immunization of mice with sheep red blood cells (SRBC)

Sheep red blood cells (Division of Animal Care Services, University of Kentucky) were washed twice and resuspended in RPMI-1640 (Microbiological Associates, Bethesda, MD) prior to use. Mice were immunized with 5×10^8 SRBC via tail vein inoculation.

On Days 3–7, following immunization, mice were killed by cervical dislocation, the spleens removed, teased into a dispersed cell suspension in RPMI-1640 medium, and washed. The number of nucleated spleen cells was determined by counting in a haemocytometer and adjusted to 5×10^6 cells/ml in RPMI-1640 medium. The number of IgM and IgG antibody-forming cells was quantitated using a modification (Elliott & Roszman, 1975) of the Cunningham plaque-form-

ing cells (PFC) assay (Cunningham & Szenberg, 1968). For visualization of indirect (IgG) PFC, rabbit anti-mouse IgG serum (heavy and light chains, Cappel Laboratories, Cochranville, PA) was used in the assay.

Statistical analysis

Data presented as mean ± SEM were analysed by the two-tailed Student's *t*-test for independent means.

RESULTS

Effect of 5-HTP on the primary antibody response to SRBC

Studies were performed to determine the effects of 5-HTP, which increases serotonin biosynthesis (Wurtman & Fernstrom, 1976), on the magnitude and kinetics of the primary antibody response to SRBC. The results in Fig. 1 demonstrate that administration of 5-HTP has no effect on the kinetics of either the IgM or IgG PFC response. The IgM response reaches a maximum on Day 4 after immunization, and the IgG response on Day 6 in both 5-HTP treated and control animals. A marked suppression of IgG PFC was observed on Day 6 of the response in animals treated with 5-HTP. A small but significant ($P < 0.05$) decrease in IgM PFC was also noted in these animals.

Mice were inoculated with various concentrations of 5-HTP and the IgM and IgG PFC quantitated on Days 4 and 6, respectively, after immunization with SRBC. The results in Fig. 2 demonstrate that both the IgM and IgG PFC responses were suppressed in a dose-dependent manner by 5-HTP. While both the IgM and IgG responses were maximally suppressed by 200 mg/kg of 5-HTP, significant ($P < 0.01$) suppression of the IgG response occurred at 50 mg/kg, and of the IgM response at 200 mg/kg.

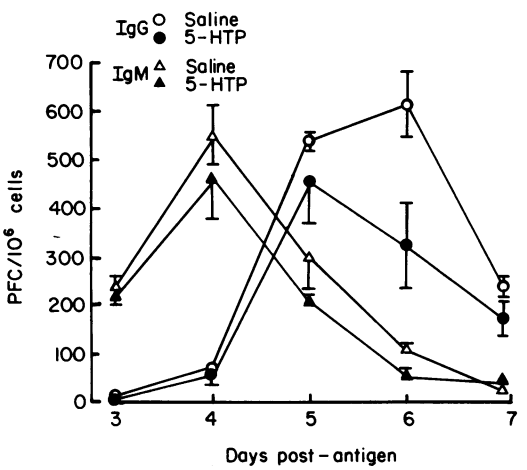

Figure 1. Effect of 5-HTP on the primary IgM and IgG response to SRBC. Mice were inoculated with 100 mg/kg 5-HTP or saline 30 min prior to immunization with 5×10^8 SRBC (Day 0). Results represent the mean number of IgM and IgG PFC/10^6 spleen cells ± SEM. Four to five animals were employed at each day of the response.

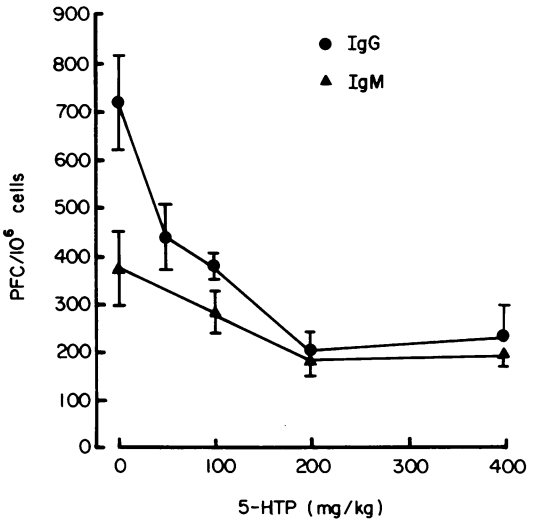

Figure 2. Effect of 5-HTP concentration on the primary IgM and IgG response to SRBC. Mice were inoculated with 5-HTP (50–400 mg/kg) or saline 30 min before immunization with 5×10^8 SRBC (Day 0). Splenic IgM and IgG PFC were quantitated on Day 4 and Day 6 after immunization, respectively. Results represent the mean number of IgM and IgG PFC/10^6 spleen cells ± SEM. Four to five animals were employed at each day of the response.

The effect of PCPA on the primary antibody response to SRBC

Having observed suppression of the primary antibody response to SRBC by 5-HTP, we determined the effect of PCPA on the response. Administration of PCPA prevents the biosynthesis of serotonin by inhibiting the rate-limiting enzyme tryptophan hydroxylase (Koe & Weissman, 1966), thus resulting in decreased levels of serotonin. The data presented in Fig. 3 indicate that PCPA does not alter the kinetics of the response, but does increase the number of IgM PFC to 160% of control values on Day 4 after immunization. Similarly, the IgG PFC response is increased in PCPA-treated

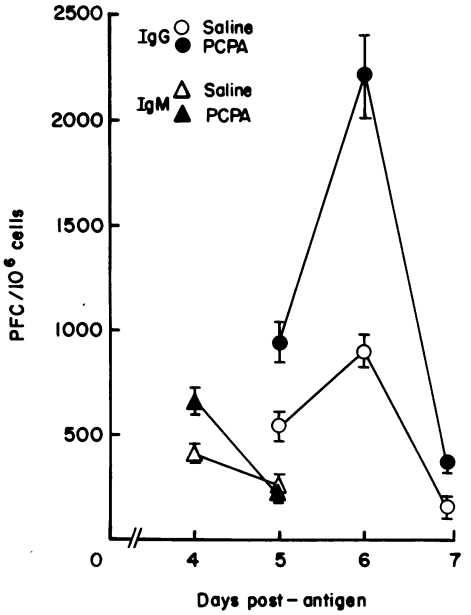

Figure 3. Effect of PCPA on the primary IgM and IgG response to SRBC. Mice were inoculated with 250 mg/kg PCPA or saline 48 hr before immunization with 5×10^8 SRBC. Results represent numbers of IgM and IgG mean PFC/10^6 spleen cells ± SEM. Four to five animals were employed at each day of the response.

animals on Days 5–7 after immunization, with increases of 177% and 250% above control values on Days 5 and 6, respectively.

The effect of serotonin on the primary antibody response to SRBC

Data obtained with the drugs 5-HTP and PCPA suggest that serotonin can modulate the primary antibody response to SRBC. In order to test this directly, animals were inoculated with serotonin and, 30 min later, immunized with SRBC. The results in Table 1 demonstrate systemic administration of serotonin produces a highly significant suppression of the IgM ($P < 0.001$) and IgG ($P < 0.01$) responses. To further examine this suppression, groups of animals were inoculated with 0·08–100 mg/kg of serotonin and, 30 min later, immunized with SRBC. Suppression of both the IgM and IgG responses was observed with as little as 0·4 mg/kg of serotonin (Fig. 4).

Table 1. The effect of serotonin on the IgM and IgG primary antibody response to SRBC

Treatment*	IgM PFC†	IgG PFC†
Serotonin (100 mg/kg)	144 ± 14‡	274 ± 54‡
Saline	251 ± 6	506 ± 14

* Mice were immunized with SRBC 30 min after inoculation with either serotonin or saline. Five animals were employed in each treatment group.

† Results represent PFC/10^6 nucleated spleen cells ± SEM. IgM and IgG PFC were determined on Days 4 and 6, respectively, after immunization.

‡ Significant values derived by comparing results from serotonin treated animals to saline control group ($P < 0.01$).

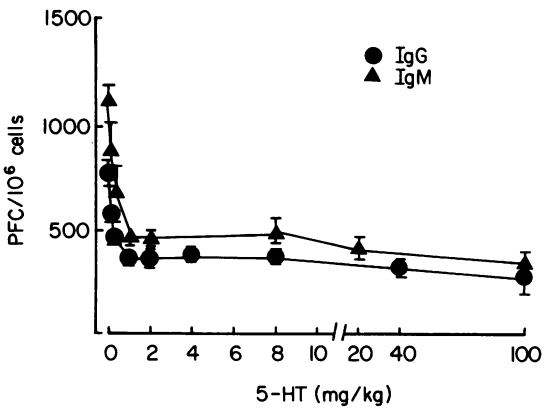

Figure 4. Effect of serotonin on the primary IgM and IgG response to SRBC. Mice were inoculated with saline or 5-HT (0·08–100 mg/kg) 30 min before immunization with 5×10^8 SRBC (Day 0). Splenic IgM PFC were quantitated on Day 4 and IgG PFC on Day 6 after immunization. Results represent the mean number of IgM and IgG PFC 10^6 spleen cells ± SEM. Four to five animals were employed at each day of the response.

Effect of intracisternal injection of the neurotoxin 5,7-DHT on the primary antibody response to SRBC

Experiments were performed to determine if altering the concentration of serotonin in the brain resulted in modulation of the primary antibody response. Animals were inoculated intracisternally with 5,7-DHT, a neurotoxin which effects serotonin- and catecholamine-containing neurons, and immunized with SRBC. The results in Table 2 demonstrate that 5,7-DHT treated animals have a decreased number of IgM and

Table 2. The effect of intracisternal injection of 5,7-DHT or 5,7-DHT plus DMI on the IgM and IgG antibody response to SRBC and on brain serotonin levels

Treatment*	IgM PFC†	IgG PFC†	Serotonin pg/mg wet weight‡
5,7-DHT	87±21§**	371±11***	81±15**
5,7-DHT+DMI	314±125 (NS)	1382±149 (NS)	93±2**
Saline	427±80	1544±181	242±58

* Mice were injected with SRBC 48 hr after intracisternal injection of drugs. Five animals were employed in each treatment group.

† Results represent PFC/10^6 nucleated spleen cells±SEM. IgM and IgG PFC were determined on Days 4 and 6, respectively, after immunization.

‡ Serotonin concentrations were determined in brain homogenates by a radioenzymatic assay.

§ Significance derived by comparing results from the drug treatment groups to saline control group.

** $P<0.05$.

*** $P<0.001$.

IgG PFC. As 5,7-DHT can affect both serotonin- and catecholamine-containing neurons, animals were pretreated with DMI to spare catecholamine-containing neurons upon intracisternal injection of 5,7-DHT. The number of IgM and IgG PFC generated in these animals to SRBC was not significantly different from normal values (Table 2). Treatment with DMI alone did not influence the antibody response to SRBC (data not shown). Animals receiving 5,7-DHT, whether alone or subsequent to DMI pretreatment, had markedly depleted brain serotonin levels, as compared to control animals (Table 2).

Effect of 5-HTP, PCPA and serotonin on serum corticosterone concentrations

Alterations in the concentration of serotonin via drug treatments can affect plasma levels of corticosterone (Fuller & Snoddy, 1980), therefore, levels of this steroid were quantitated in sera of mice treated with either 5-HTP, PCPA or serotonin. The results in Fig. 5 demonstrate that 5-HTP, PCPA and serotonin all elevate plasma corticosterone levels at 1 hr post-injection, with a return to control values by 6 hr. The largest increase was observed with 5-HTP, whereas smaller increases were noted with 0·4 mg/kg serotonin and PCPA. At 3 hr after drug treatment, animals inoculated with 0·4 mg/kg serotonin were at control values.

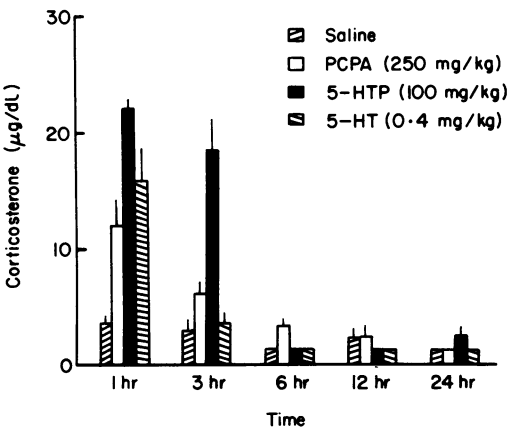

Figure 5. Effect of PCPA, 5-HTP and serotonin on plasma corticosterone. Mice were inoculated with either saline, PCPA (250 mg/kg), 5-HTP (100 mg/kg), or 5-HT (0·4 mg/kg) at 09.00 hr. They were killed by exsanguination 1, 3, 6, 12 or 24 hr after drug treatment, and plasma corticosterone values determined by radioimmunoassay. Mean plasma corticosterone (μg/dl)±SEM are shown for five animals per treatment.

DISCUSSION

The present report indicates that serotonin has immunomodulatory properties. These results establish that serotonin is capable of modulating both the IgM

and IgG primary antibody response to SRBC. The administration of serotonin, or its precursor, 5-HTP, significantly impairs the antibody response, whereas inhibition of serotonin synthesis by PCPA markedly enhances the response. Although injection of serotonin equally affects both the IgM and IgG PFC response, 5-HTP and PCPA preferentially affect the IgG response. This differential effect may be explained, in part, by the fact that 5-HTP and PCPA readily cross the blood-brain barrier, whereas serotonin has limited penetration into the brain. Thus, it may be suggested that serotonin modulates lymphocyte reactivity in the periphery where the effects are direct and more profound, compared with those of 5-HTP and PCPA which may be mediated, in part, through the central nervous system and thus may be less direct. Variations in these responses also may reflect differences in sensitivity to serotonin in T-helper cells or in the PFC precursor population, the IgG-secreting lymphocyte being more susceptible to serotonin modulation than are IgM precursors.

Based on the finding that nucleus raphe ablation is associated with suppression of humoral responsiveness, and that this suppression can be subsequently abrogated by hypophysectomy (Devoino *et al.*, 1975), it has been suggested that the effects of serotonin are mediated via a central serotonergic hypothalamia-pituitary pathway. Thus, serotonin is hypothesized to modulate immune reactivity via regulation of a neurohormone or peptide which, in turn, alters lymphocyte function. The reports that serotonin remains suppressive in adrenalectomized animals (Bliznakov, 1980) indicate that corticosterone does not play a significant role in this regulatory system. In our studies, all animals that were injected with either serotonin, 5-HTP or PCPA were found to have elevated corticosterone levels. Nevertheless, the varying immunological effects of these substances (i.e. inhibition with serotonin and 5-HTP and enhancement with PCPA) indicate that alterations in primary antibody response are most consistent with changes in serotonin levels, rather than resulting from the immunosuppressive effects of corticosterone. Although hormones may be involved in this model, it is evident that the observed effect is not due to the immunosuppressive effects of corticosterone. The interrelationship of other hormones under the influence of serotonergic pathways (e.g. growth hormone, prolactin, thyrotropin-releasing hormone, and gonadotropins) remains to be investigated.

Data obtained in the present study reveal that alteration of the serotonin content of the brain is not associated with impairment of the antibody response to SRBC. Thus, specific destruction of central serotonergic neurons after intracisternal injection of 5,7-DHT into animals which have been pretreated with DMI (which blocks the uptake in catecholamine neurons) has no effect on the PFC response to SRBC. Intracisternal administration of 5,7-DHT alone, which destroys both serotonin- and catecholamine-containing neurons (Björklund *et al.*, 1974), however, did result in suppression of PFC responses. These data suggest that the immuno-modulatory effects of serotonin are not mediated through neural pathways in the brain, but rather through peripheral mechanisms directed toward the lymphocyte itself. Further support for this hypothesis is gained from the finding that systemically-administered serotonin, which has extremely low penetration through the blood-brain barrier, is associated with a marked suppression of PFC generation, hence a peripheral effect. As a corollary, it may be anticipated that receptors for serotonin, similar to those for adrenergic substances (Loveland *et al.*, 1981; Pochet *et al.*, 1979; Watanabe *et al.*, 1981), will be present on the surface of the lymphocyte. Although preliminary investigations indicate that lymphocytes have high affinity S_1 serotonin receptors and macrophages lower affinity S_2 receptors (Roszman *et al.*, 1984), further study is required to establish the nature of these receptors and their presumed immunomodulatory role. Data obtained from the neurotoxin studies indicate that, although the effects of serotonin are not mediated through central neural pathways, there are catecholamine interconnections in the brain which are important in immune regulation. This observation is supported by the reports of Besedovsky *et al.* (1983b) which demonstrate that changes in hypothalamic catecholamine levels occur during the generation of immunity. Thus, the data would indicate that neurotransmitters are capable of influencing lymphocyte function by peripheral and central neural pathways, and are thereby mediators of neuroimmunomodulation.

In summary, this study demonstrates that alterations in serotonin are capable of modulating immunoglobulin secretion through mechanism(s) yet to be elucidated. It is evident, however, that this neurotransmitter directly effects lymphocyte function, rather than influencing immune responsiveness through serotonergic pathways in the central nervous system.

ACKNOWLEDGMENTS

This work was supported, in part, by N.I.H. grants NS-17423 and AG 02435.

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