Immunology 1986 **57** 145–152

Neuroimmunomodulation: impairment of humoral immune responsiveness by 6-hydroxydopamine treatment

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Accepted for publication 18 September 1985

SUMMARY

Previous studies from this laboratory and others show that perturbations of the central nervous system modulate immune function. In addition, reports from several investigators indicate that depletion of the neurotransmitter norepinephrine (NE) in peripheral nerves by injecting the neurotoxin 6-hydroxydopamine (6-OHDA), can enhance or suppress the antibody response. However, immunocompetence following brain depletion of catecholamines has not been investigated. In this study, we investigated the effects of injecting 6-OHDA into the cisterna magna of male CBA/J mice, and determined the effects of this treatment on both the IgM and IgG antibody responses to sheep red blood cells (SRBC). Both responses are suppressed compared to saline-injected control or normal animals. Animals treated with 6-OHDA have decreased levels of NE in the midbrain, pons-medulla and hypothalamus, while dopamine levels did not change in these brain regions but was decreased in the striatum. The percentage of splenic T cells and B cells was not affected by 6-OHDA treatment. Although there is a marked increase in plasma corticosterone levels in 6-OHDA-treated mice, saline-injected control animals have equivalent increases in plasma corticosterone without concomitant impairment of the immune response. Thus, the decline in immune responsiveness following 6-OHDA treatment does not result from corticosterone-induced immunosuppression. Analysis of the kinetics of the primary IgG response following 6-OHDA treatment indicates that the magnitude, but not the kinetics, of the response decreases. Experiments to determine the effects of 6-OHDA on the afferent and efferent phrases of the response demonstrate that it is effective only when administered prior to immunization, and thus must inhibit early events involved in the initiation of the response. Additional experiments show that mice can be immunized 2 weeks following brain catecholamines depletion and still exhibit a decreased antibody response. However, the response returns to normal levels if immunization is delayed 4 weeks after injection. Further experiments demonstrated that 6-OHDA treatment has no effect on the secondary antibody response, but does inhibit the development of immunological memory. Collectively, these results indicate that 6-OHDA treatment has a profound inhibitory effect on the induction of the primary antibody response and immunological memory development, but is without effect on the secondary antibody response. The data further substantiate the existence of a link between the brain and the immune response.

INTRODUCTION

The immune system has conceptually evolved as consisting of a repertoire of cells, their soluble cytokines and immunoglobulins, which function in a network of facilitory and inhibitory signals to maintain immunological homeostasis. Jerne (1974) has compared the immune system with the central nervous system; each is capable of responding to an unlimited number of external as well as internal stimuli, and of displaying adaptive behaviour based on experience and memory. Hence, modulation within these networks represents the organism's attempt to interact appropriately with the external environment. More recently, it has become evident through a variety of investigative (Cross *et al.*, 1980; Brooks *et al.*, 1982; Besedovsky *et al.*, 1979; Jankovic & Isakovic, 1973) and clinical (Stein, Schiavi & Camerino, 1976; Bartrop *et al.*, 1977; Solomon, 1969) correlates that these seemingly unrelated systems are indeed intricately linked in a concept of neuroimmunomodulation.

Although it is well established that ablation of specific areas in the brain is associated with dramatic alterations in immune functions (Cross *et al.*, 1980; Jankovic & Isakovic, 1973; Cross *et*

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al., 1984; Goldstein, 1976; Lupparello, Stein & Park, 1964; Macris *et al.*, 1970), the one or more mechanisms involved in brain–immune interaction remain to be explored. The finding of receptors for hormones (Arrenbrecht, 1974; Harrison, Flier & Itin, 1979; Wang *et al.*, 1984) and neurotransmitters (Williams, Synderman & Lefkowitz, 1976; Loplor *et al.*, 1980; Hozum, Chang & Cautrecasas, 1979; Hohlfield *et al.*, 1984) on the surface membrane of the lymphocyte suggests that these soluble mediators are the communicative link. Besedovsky *et al.* (1979), Del Rey *et al.* (1981) and Miles *et al.* (1981) have reported that depletion of norepinephrine (NE) in the spleen by sympathetic axotomy results in increased humoral responsiveness. Recently, we have demonstrated that serotonin, a central and peripheral neurotransmitter, has significant facilitory and inhibitory properties on immune function (Jackson *et al.*, 1985). These effects, however, occur in the periphery at the level of the lymphocyte or monocyte, or both, and are not mediated through alterations of serotonergic pathways in the brain. Thus, although it is evident that neurotransmitters can change immune reactivity, it has not been shown that these effects result from alterations in the specific chemical neuroanatomy of the brain, namely, altered function of neurotransmitter-containing neurons.

The present investigation extends the concept of neuroimmunomodulation by demonstrating that injection of 6-hydroxydopamine (6-OHDA) into the cisterna magna results in marked inhibition of the antibody response to sheep red blood cells (SRBC). This modulation of the antibody response is not related to changes in circulating corticosterone, nor shifts within specific peripheral lymphocyte subpopulations. This study, furthermore, indicates that the immunological effects of catecholamine depletion affect the afferent limb of the immune response, including the development of memory cells. Thus, it may be important for maintaining immunological integrity, adding credence to the concept of a homeostatic relationship between the brain and immunity.

MATERIALS AND METHODS

Animals

Male CBA/J mice (Jackson Laboratories, Bar Harbor, ME) aged 55–70 days were used in these studies.

Reagents

SRBC were obtained from one animal maintained by the Division of Lab. Animal Resources, University of Kentucky. 6-Hydroxydopamine, a catecholamine neurotoxin, was obtained from Sigma Chemical Co., St Louis, MO.

Depletion of CNS catecholamines

Mice were anaesthetized with Metofane (methoxyflurane, Pitman-Moore, Washington Crossing, NJ) and injected into the cisterna magna with 100 μg of 6-OHDA dissolved in sterile saline in a volume of 10 μl using a 26-g needle. Animals were injected within 5 min of 6-OHDA preparation. Saline-injected and uninjected animals served as controls, and the immune response of these two groups did not differ significantly.

Corticosterone assay

Corticosterone was measured using a [^{3}H]corticosterone assay kit purchased from Radioimmunoassay Systems Laboratories, Carson, CA. Saline or 6-OHDA were injected into the cisterna magna between 0800 hr and 0900 hr. Plasma was collected in tubes containing ethylene diamine tetra-acetate (EDTA) at various times after drug injection. Uninjected animals were also sampled at each interval to control for changes in plasma corticosterone due to diurnal rhythms. Levels are expressed as ng corticosterone/ml of plasma.

Quantification of brain neurotransmitters

Mice were injected with 6-OHDA or saline as described. Two days later, the animals were decapitated, the brain quickly removed, and the hypothalamus, striatum, midbrain and a section of medulla-pons dissected by the method of Glowinski & Iverson (1966) on an ice-chilled glass block. Levels of dopamine (DA) and NE in brain samples were determined by the methods of Sparks & Slevin (1985). Brain tissue was weighed, sonicated in six vols (w/v) of 0·1 M perchloric acid, centrifuged at 51,000 ***g*** for 10 min, and the supernatant injected onto the liquid chromatography (LC) column. Whole heart was weighed, sonicated in six vols (w/v) of 0·1 M perchloric acid, resonicated after the addition of 500 μl of high pressure liquid chromotography grade chloroform, centrifuged at 51,000 ***g*** for 10 min, and the aqueous supernatant injected onto the LC column. There is essentially 100% recovery of DA and Ne from the heart using this method. Neurochemicals were detected electrochemically (BAS West Lafayette Ind.) and quantified by peak height based on external standards obtained from the Sigma Chemical Company.

Immunization and plaque-forming cell (PFC) assay

Mice were injected with 5×10^8 SRBC intravenously (i.v.) at various times before or after 6-OHDA treatment. Plaque-forming cells (PFC) were quantified using the slide chamber method of Cunningham & Szenberg (1968). Indirect (IgG) PFC were developed using a 1:50 final dilution of goat anti-mouse IgG (Cappel Labs, Cochranville, PA). The data are reported as PFC/10^6 nucleated spleen cells.

Immunofluorescent staining

Direct staining methods were used to enumerate splenic B cells and T cells following 6-OHDA treatment. B cells were stained using a fluorescein (FITC)-conjugated F(ab′)$_2$ fragment of a goat anti-mouse IgG antibody, heavy and light chain-specific (Cappel Labs). T cells were stained using an FITC-conjugated mouse anti-Thy 1.2 monoclonal antibody (Becton-Dickinson Monoclonal Center, Mountain View, CA). Immunofluorescence was analysed on a FACS 420 fluorescence activated cell sorter (Becton-Dickinson FACS Systems, Sunnyvale, CA).

Statistical analysis

Data were analysed using Student's two-tailed *t*-test for independent means. Each experiment was repeated at least three times.

RESULTS

Effect of 6-OHDA on the primary SRBC antibody response

Experiments were conducted to determine the effect of cisterna magna injection of 6-OHDA on the IgM and IgG PFC response. Initially, animals were injected with 6-OHDA 2 days prior to immunization with SRBC and the ensuing IgM and IgG response determined (Table 1). These results clearly demon-

Table 1. Effect of intracisternal injection of 6-OHDA on the primary *in vivo* antibody response to SRBC

Treatment*	Antibody response† (PFC/10^6 nucleated cells) IgM	IgG
6-OHDA	119±12‡ ($P<0.02$)	870±226 ($P<0.05$)
Saline	200±25	1720±161
Uninjected control	259+20	ND§

* Mice were immunized 48 hr following intracisternal injection of 6-OHDA or saline. There were four animals in each treatment group.

† IgM and IgG PFC were enumerated on Days 4 and 6, respectively, after immunization.

‡ Results represent the mean PFC/10^6 cell-±SEM values obtained from 6-OHDA-treated animals were compared to values from saline-injected controls employing Student's *t*-test. The saline-injected controls did not differ significantly from normal animals.

§ ND, not determined.

strate that animals injected with 6-OHDA have a decrease in the number of both IgM and IgG PFC as compared to control animals. Moreover, the IgG PFC response in animals injected intraperitoneally with 100 μg 6-OHDA did not differ from saline-injected animals (data not shown); hence, the immunological effects of 6-OHDA result from central, but not peripheral,

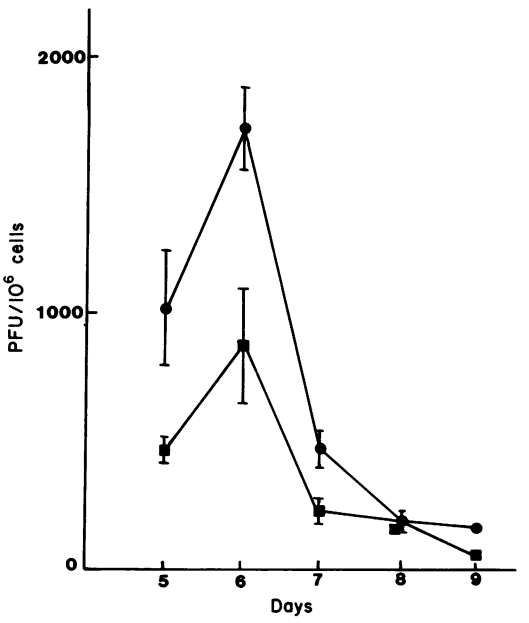

Figure 1. The effect of 6-OHDA on the kinetics of the IgG PFC response. Mice were injected with 6-OHDA (■) or saline (●). Two days later, the mice were immunized with SRBC and the IgG PFC response determined. The response of 6-OHDA-injected mice is significantly depressed on Days 5, 6, and 7 ($P<0.05$). Each point represents the mean of five animals.

Table 2. Effect of the interval between 6-OHDA treatment and immunization on the primary immune response to SRBC

Interval between 6-OHDA and immunization*	Treatment of animals: Saline PFC/10^6 cells)†	6-OHDA (PFC/10^6 cells)†
2 weeks	550±106‡ (4)	107±56 (4) ($P<0.005$)
4 weeks	563±48 (4)	540±62 (4) (NS)§

* Animals inoculated with 100 μg of 6-OHDA and immunized with 5×10^8 SRBC (i.v.).

† Number of indirect (IgG) PFC determined 6 days after immunization.

‡ Results represent the mean±SEM. Numbers in parentheses are the number of animals per group.

§ NS, not significant.

catecholamine depletion. In order to examine further the effect of central catecholamine depletion, animals were injected with 6-OHDA as previously described, and the kinetics of the primary IgG antibody response determined (Fig. 1). Although the response of 6-OHDA-treated animals was decreased in magnitude during the peak of the normal SRBC response (Days 5–7), there was no alteration in the kinetics of this response.

Additional experiments were initiated to determine the longevity of the effects of 6-OHDA on the IgG response. Mice were injected with 6-OHDA, then immunized with SRBC either 2 or 4 weeks after injection of 6-OHDA or saline into the cisterna magna. The primary IgG response was determined on Day 6 after immunization. The data in Table 2 indicate that these effects persist for 2 weeks; however, the response returns to normal levels by the fourth week following the 6-OHDA treatment.

The effects of 6-OHDA treatment on brain catecholamine concentrations, serum corticosterone concentration and splenic T- and B-cell percentage

In order to verify that 6-OHDA is specific in its action, the concentrations of NE and DA in the hypothalamus, striatum, midbrain and pons-medulla were measured 48 hr after 6-OHDA injection. The results in Table 3 demonstrate that 6-OHDA treatment depletes the hypothalamus, midbrain and pons-medulla of NE. The concentration of DA did not change in the hypothalamus, midbrain or pons-medulla after the injection of 6-OHDA, but did decrease in the striatum as compared to values obtained with non-injected and saline-injected animals.

Control experiments were also performed to measure the effect of cisterna magna injection of 6-OHDA on peripheral tissues. These data show that the NE content in the heart did not differ in the 6-OHDA-injected or control animals 2 days following 6-OHDA treatment: 6-OHDA 0·822±0·033 ng/mg compared to 0·820±0·007 ng/mg in saline-injected controls and 0·734±0·0515 in normals. Thus, injection of 6-OHDA into the cisterna magna did not affect NE levels at peripheral tissue sites, only in the CNS.

One consequence of 6-OHDA treatment is that an increase

Table 3. Levels of aromatic neurotransmitters in the CNS of naive CBA mice or in mice injected with saline or 6-HDA in the cisterna magna

	Hypothalamus		Midbrain	
Treatment*	Norepinephrine	Dopamine	Norepinephrine	Dopamine
Uninjected (4)	1·22 + 0·123†	0·62 ± 0·09	1·01 ± 0·10	0·41 ± 0·02
Saline (4)	1·28 ± 0·071	0·57 ± 0·06	0·998 ± 0·05	0·35 ± 0·02
6-OHDA (4)	0·468 ± 0·071 ($P < 0·001$)‡	0·65 ± 0·07 (NS)§	0·203 ± 0·023 ($P < 0·001$)	0·31 ± 0·04 (NS)
	Striatum		Pons-medulla	
	Norepinephrine	Dopamine	Norepinephrine	Dopamine
Uninjected	1·54 ± 0·20	15·6 ± 0·55	0·404 ± 0·060	0·072 ± 0·020
Saline	1·41 ± 0·09	16·3 ± 0·96	0·444 ± 0·030	0·065 ± 0·018
6-OHDA	1·05 ± 0·17 (NS)	5·8 ± 1·12 ($P < 0·001$)	0·150 ± 0·029 ($P < 0·001$)	0·066 ± 0·018 (NS)

* Mice were injected into the cisterna mgna with 6-OHDA or saline 48 hr prior to killing. There were five animals in each treatment group.

† ng/mg wet tissue weight ± SEM; each group represents a minimum of five animals/treatment.

‡ Significance interval using Student's *t*-test comparing the 6-OHDA- and saline-injected groups.

§ NS, not significant.

Table 4. Plasma corticosterone levels after cisterna magna injection of 6-OHDA or saline

	Time after injection (hr)*					
Treatment	1	3	6	12	24	48
6-OHDA	190* ± 52	114 ± 16	94 ± 34	56 ± 14	34 ± 10	31·5 ± 7
Saline	220 ± 15	151 ± 22	92 ± 54	78 ± 31	55 ± 28	24 ± 2
None (uninjected)	23 ± 8·4	49 ± 14	28 ± 14	36 ± 8	21 ± 5	36 ± 10

* Blood was obtained beginning at 0900 hr. Each timepoint represents the mean of five animals.

in serum corticosterone can occur which may secondarily alter immunological reactivity (Cuello, Shoemaker & Ganong, 1974). We therefore measured plasma corticosterone concentration in mice at various intervals after 6-OHDA treatment. The results in Table 4 show a rapid increase in corticosterone 1 hr after 6-OHDA injection, which gradually returns to normal values within 12 hr after injection. A similar rise and fall in plasma corticosterone concentrations was also noted in control animals injected with saline. It is important to emphasize that the PFC response of animals injected with saline is not different from control animals, indicating that 6-OHDA-induced immunosuppression is not simply the result of increased concentrations of plasma corticosterone. Another possible explanation for the effects of 6-OHDA on the immune response is that this treatment alters the percentage of splenic T or B cells. Analysis of the percentage of these cells in the spleen of animals indicated that 6-OHDA-treated animals had 64 ± 1% T cells and 43 ± 2% B cells, while control animals had 62 ± 2% T cells and 38 ± 2% B cells. Similar results were also obtained 6 days after 6-OHDA treatment. Additionally, no change in the number of splenic nucleated cells was observed at any test time after this treatment (data not shown).

Effect of 6-OHDA treatment on the efferent and afferent phases of the primary SRBC antibody response

Having demonstrated that the injection of 6-OHDA into animals 48 hr prior to immunization results in suppression of the SRBC antibody response, further studies were designed to assess the effects of 6-OHDA at various times before and after immunization. Animals were injected with 6-OHDA at intervals either before or after immunization; the results are shown in Table 5. When 6-OHDA was injected 2 hr before, or 24 or 48 hr after immunization, no effect on the IgG PFC response was observed.

Effect of 6-OHDA treatment on the secondary SRBC antibody response

The effects of 6-OHDA were next extended to determine the

Table 5. Effect on the primary SRBC antibody response of treating animals with 6-OHDA before or after immunization

	Treatment of animals	
6-OHDA injected before or after immunization*	Saline (PFC/10^6 cells)†	6-OHDA (PFC/10^6 cells)†
2 hr before	1170±119‡	1480±260 (NS)§
24 hr after	913±133	918±117 (NS)
48 hr after	672±61	926±61 (NS)

* Animals inoculated into the cisterna magna with 100 μg of 6-OHDA and immunized with 5×10^8 SRBC. There were five animals in each treatment.

† Number of indirect (IgG) PFC determined 6 days after immunization.

‡ Results represent the mean ± SEM.

§ NS, not significant.

effects of this treatment on the secondary SRBC antibody response. Animals were immunized with SRBC, 2 weeks later injected with 6-OHDA, and 48 hr later reimmunized with SRBC. The results in Fig. 2 demonstrate that 6-OHDA has no effect on either the magnitude or kinetics of the secondary IgG PFC response.

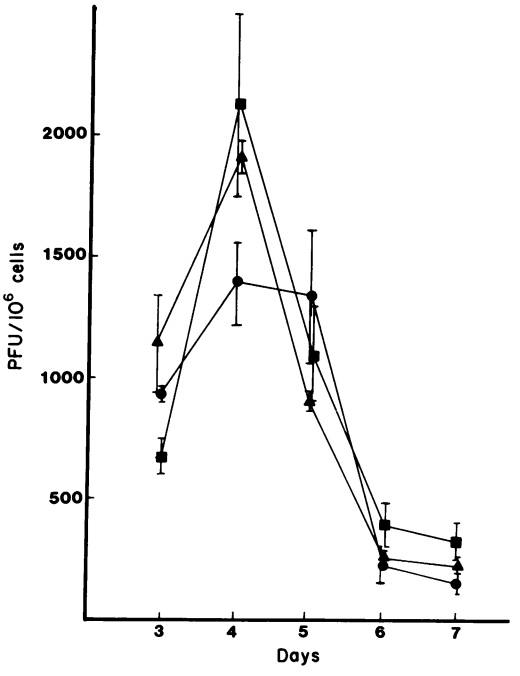

Figure 2. The effect of 6-OHDA treatment on the secondary IgG response. Mice were primed with SRBC, rested 2 weeks, then injected with 6-OHDA (■), saline (●), or received no injection (▲). Two days after drug treatment, all mice were reimmunized and the IgG PFC response determined. The response of all groups did not differ significantly. Each point represents the mean of five animals.

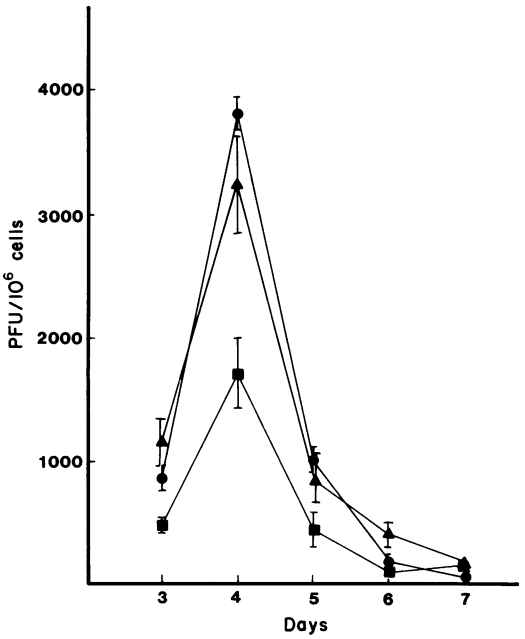

Figure 3. The effect of 6-OHDA on the development of immunological memory. Animals were injected with 6-OHDA (■) or saline (●), and 2 days later these drug-treated mice plus an uninjected (▲) control group were immunized with SRBC. Two weeks later, all of the mice were reimmunized and the IgG PFC response determined. The response of 6-OHDA-treated animals was significantly different from that of control animals (Day 3, $P < 0.01$; Day 4, $P < 0.001$; and Day 5, $P < 0.05$). Each point represents the mean of five animals.

Effect of 6-OHDA treatment on the development of immunological memory

In order to determine if the development of immunological memory is influenced by 6-OHDA, animals were injected with the drug 48 hr prior to primary immunization with SRBC. Fourteen days later, the animals were reimmunized with SRBC and the IgG PFC response determined. As shown in Fig. 3, 6-OHDA treatment decreased the magnitude of the IgG PFC response as compared to control animals, but had no effect on the kinetics of the response. Thus, whereas central catecholamine depletion has no effect on the secondary antibody response, the primary response and development of immunological memory are dependent on the integrity of catecholamine-containing neurons in the brain.

DISCUSSION

The present studies demonstrate that 6-OHDA treatment of animals has a differential effect on the ensuing immune response to SRBC. Animals treated with 6-OHDA have a marked decrease in their ability both to elicit a primary SRBC antibody response and to develop immunological memory. The subsequent secondary antibody response is resistant to this treatment, suggesting that memory cells are not subject to the same neural modulation in this system as are virgin immunocompetent cells. The data further demonstrate that 6-OHDA treatment affects early immunological events, as administration of 6-OHDA after immunization is without effect. These cumulative

data indicate that the brain catecholamine content is important in the afferent limb of the immune response, and provides evidence to suggest that neurochemical alterations of the brain modulate immune function.

Treatment of animals with 6-OHDA resulted in significant decreases in NE in the hypothalamus, midbrain and pons-medulla, while DA was decreased only in the striatum. These data are in general agreement with previous observations of the effects of intraventricular injection of 6-OHDA on brain catecholamine levels in the rat (Bell, Iverson & Uretsky, 1970; Kostrzewa & Jacobowitz, 1974). The indication that the effects we have observed with 6-OHDA are confined to the brain is supported by the fact the NE is not affected in peripheral tissue, and that the peripheral injection of 100 μg of 6-OHDA has no effect on the primary SRBC antibody response.

Although this study represents the initial exploration into the effects of neurochemical alterations in the brain and subsequent immune reactivity, the possible role of catecholamines as a mediator of neuroimmunomodulation has been reported by others (Besedovsky *et al.*, 1979; Del Rey *et al.*, 1981; Miles *et al.*, 1981; Williams *et al.*, 1981). These previous investigations, however, have been directed primarily at the peripheral effects of 6-OHDA treatment. Besedovsky *et al.* (1979), using multiple intraperitoneal injections of 6-OHDA, demonstrated that spleen NE depletion resulted in an enhanced antibody response to SRBC only when coupled with adrenalectomy. This study is in contrast to those of Hall *et al.* (1982) and Kasahara *et al.* (1977) who reported that peripheral catecholamine depletion has an inhibitory influence on this humoral response. Miles *et al.* (1981) reported that peripheral injections of 6-OHDA resulted in suppression only of thymus-independent responsiveness. It should be emphasized that these studies utilize peripherally injected 6-OHDA to achieve catecholamine depletion. Because 6-OHDA does not cross the blood–brain barrier to any appreciably degree in adult animals (Cuello *et al.*, 1974), the immunological effects of peripheral catecholamine depletion, albeit conflicting, are not strictly comparable to those of the current study.

Because of the influence of hypothalamic-releasing factors on the maintenance and balance of peripherally circulating hormones, it is possible that the effects of catecholamine depletion are related to changes in pituitary release of ACTH and subsequent elevation of circulating corticosterone, which have been shown to suppress lymphocyte function (Vischer, 1972). Corticosterone blood levels were similarly elevated in both experimental groups yet inhibition of the SRBC response was found only in those that received 6-OHDA. These data, therefore, indicate that the abrogation of immune function subsequent to 6-OHDA treatment is not simply the result of increased corticosterone secretion induced by surgically induced stress (Plumpton, Besser & Cole, 1964). Although the effects of decreasing central catecholamine levels are not mediated via the pituitary–adrenal axis, the hypothesis that hormones are the mediators of neuroimmunomodulation remains attractive. Circulating lymphocytes have been shown to contain a variety of surface membrane receptors for hormones (Arrenbrecht, 1974; Harrison *et al.*, 1979; Russell *et al.*, 1985). Many of these hormones are capable of modifying lymphocyte function through a variety of mechanisms: chief among these is alterations in cyclic nucleotides (Pierpaoli & Maestroni, 1978; Tsoukas, Provvredini & Manolagas, 1984; O'Hearn & Stites, 1983; Gupta, Fikrig & Noral, 1983). These data suggest that the endocrine influence of immune responsiveness is operant at the earliest events of lymphocyte reactivity. Extrapolating these observations with those of the present study, it may be suggested that the immunological effects of catecholamine decreases (i.e. suppression of the afferent loop of responsiveness) are manifest via alterations in hypothalamic-releasing factors, and hence fluxes in circulating hormones. Indeed, central catecholamine reduction does transiently affect the secretion of prolactin, growth hormone and luteinizing hormone (Fenske & Wuttke, 1976; Willoughby & Day, 1981), all of which may be capable of altering lymphocyte activity. Thus, a model of neuroimmunomodulation begins to unfold as a network of neuro–endocrine–immune interactions.

Although it is tempting to ascribe immunoregulatory function to catecholamines in this experimental model, recent evidence suggesting that classical neurotransmitters and regulatory neuropeptides can coexist in and be released from the same neuron (Everitt *et al.*, 1984; Hokfelt, 1983; Charnay, 1982) indicates that these conclusions must be interpreted with caution. Catecholamine neurons also are capable of releasing enkephalin (Charnay, 1982), neurotensin (Hokfelt *et al.*, 1984) and neuropeptide Y (Hokfelt, 1983). Whereas these latter peptides have no known effect on lymphocyte function, the enkephalins have been shown to modulate immune function (Faith *et al.*, 1984; Mathews *et al.*, 1983). Similar to the presumed mechanism(s) of endocrine–immune interaction, the possibility that neurotransmitters or peptides or both, may play a physiological role in the maintenance of immunocompetence is supported by the presence of specific receptors for neurotransmitters and peptides on the surface membrane of lymphocytes capable of altering lymphocyte reactivity [e.g. acetylcholine (Hohlfied *et al.*, 1984), substance P (Payan, Levine & Goetzl, 1984), opiates, endorphin (Loplor *et al.*, 1980; Hozum *et al.*, 1979; Shavit *et al.*, 1984) and beta-adrenergic receptors (Miles *et al.*, 1984). Thus, these data clearly demonstrate that alterations in neurotransmitters and peptides are capable of influencing the lymphoid elements of the immune system.

These and previous data indicate that a complex network of exogenous as well as endogenous factors are involved in the maintenance of immunological homeostasis (Besedovsky, Del Rey & Sorkin, 1984). Thus, the immune system can no longer be conceived as an isolated entity; it responds to and effects changes in the central nervous and endocrine systems. The precise mechanisms by which this information is interchanged among these systems remains conjectural and to be explicated.

ACKNOWLEDGMENTS

This work was supported by U.S. Public Health Service Grants NS 17423 and AG 02435, and a grant from the University of Kentucky Research Fund.

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