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Central CRH Suppresses Specific Antibody Responses: Effects of β-Adrenoceptor Antagonism and Adrenalectomy

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Central corticotropin releasing hormone (CRH)-induced activation of the sympathetic nervous system and/or the pituitary–adrenal axis is hypothesized to mediate suppression of *in vivo* specific antibody responses. To test whether β-adrenergic receptor activation is involved in the immunosuppressive effects of central CRH, rats were pretreated with propranolol or saline before intracerebroventricular infusion of CRH and immunization with keyhole limpet hemocyanin (KLH). KLH (3 μg/kg) immunization induced significant increases in circulating levels of antigen-specific IgM and IgG. Central infusion of CRH (200 pmol) suppressed both IgM and IgG responses. Pretreatment with propranolol (20 mg/kg IP) reversed CRH-induced suppression of IgG responses but had no effect on IgM levels. To test whether adrenal activation also plays a role in the effects of KLH on specific antibody responses, a separate group of animals underwent adrenalectomy prior to CRH infusion and immunization with KLH. As compared to nonadrenalectomized control rats, adrenalectomized rats showed a reduction of antibody responses, and CRH failed to induce a further suppression of IgM or IgG responses in adrenalectomized animals. Collectively, these data suggest that β-adrenoceptors mediate the suppression of primary antibody responses induced by central CRH. Moreover, the adrenals may promote optimal primary antibody responses after exposure to physiological levels of antigen.

Key Words: corticotropin-releasing hormone; β-adrenoceptors; sympathetic nervous system; keyhole limpet hemocyanin; adrenalectomy; propranolol; *in vivo* primary antibody response; rats; stress; immunity.

INTRODUCTION

Corticotropin-releasing hormone (CRH) is released during stress and acts within the brain to coordinate visceral and behavioral responses to stress (Britton, Koob, Rivier, & Vale, 1982; Britton, Lee, Vale, Rivier, & Koob, 1986; Brown & Fisher, 1985; Sutton, Koob, LeMoal, Rivier, & Vale, 1982). Elevated concentrations of CRH in the brain are also associated with marked alterations of *in vitro* correlates of natural and cellular immune function; intracerebroventricular (ICV) infusion of CRH significantly reduces *ex vivo* measures of immune function such as mitogen-induced lymphocyte proliferation and natural killer (NK) cytotoxicity in rats (Irwin, Hauger, Brown, & Britton, 1988; Irwin, Hauger, Jones, Provencio, & Britton, 1990; Irwin, Vale, & Rivier, 1990; Jain et al. 1991; Strausbaugh & Irwin 1992). *In vivo* immune function, as measured by specific IgM and IgG production following immunization with keyhole limpet hemocyanin (KLH), is also reduced following ICV CRH administration (Irwin, 1993).

There is now considerable evidence suggesting that the sympathetic nervous system mediates many immunosuppressive effects of centrally acting CRH. Postganglionic blockade, nonselective β1-β2-, or selective β2-adrenergic receptor antagonism have been shown to abrogate the reduction of splenic NK activity following central infusion of CRH (Irwin, Hauger, Jones, Provencio, & Britton, 1990; Irwin, Hauger,

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Brown, & Britton, 1988). Moreover, urocortin, a recently identified neuropeptide that is closely related to CRH, has also been shown to suppress lymphocyte proliferation responses *in vitro* when administered ICV, and this effect is reversed by postganglionic blockade with chlorisondamine or β-adrenergic receptor antagonism with propranolol (Okamoto, Ishikawa, Kimura, & Saito, 1998). The extent to which acute sympathetic activation also regulates *in vivo* specific antibody production is unknown, although some evidence supports the hypothesis that sympathetic influence is suppressive. Kruszewska et al. (1995) have shown, for example, that chemical sympathectomy with 6-hydroxydopamine in mice enhances antigen-specific splenocyte proliferative responses *in vitro* and specific antibody responses *in vivo,* data that suggest tonic inhibition of humoral immunity by the sympathetic nervous system. In addition, Sanders and colleagues have found that β-adrenergic receptor activation *in vitro* modulates antibody production (Sanders & Powell-Oliver, 1992), possibly through effects on Th1 lymphocytes (Ramer-Quinn, Baker, & Sanders, 1997). Finally, Friedman et al. (1995) have shown that *in vivo* administration of the sympathetic neurotransmitter neuropeptide Y results in a dose-dependent suppression of specific antibody responses to KLH.

CRH effects on immune function may also be mediated by activation of the pituitary–adrenal axis. CRH potently stimulates the release of adrenocorticotropic hormone (ACTH; Owens & Nemeroff, 1991), which in turn releases corticosterone from the adrenal cortex. Acute elevations of corticosterone are thought to contribute to the suppression of lymphocyte proliferation and macrophage cytokine production following aversive footshock (Cunnick, Lysle, Kucinski, & Rabin, 1990) or central infusion of interleukin-1 (Brown et al. 1991). The glucocorticoid antagonist RU-486 has been shown to block the suppressive effects of inescapable footshock on *in vivo* antibody responses, implicating endogenous release of glucocorticoids in stress-induced suppression of humoral immunity (Fleshner, Brennan, Nguyen, Watkins, & Maier, 1996). In contrast, other studies have found that stress-induced suppression of NK activity is independent of pituitary–adrenal activation (Irwin, Vale, & Rivier, 1990).

In the present study, rats were immunized with KLH, a noninfectious antigen that elicits a vigorous, clonally diverse IgM and IgG primary antibody response requiring macrophage processing and presentation as well as T and B cell cooperation (Stenzel-Poore & Rittenberg, 1989). To assess the role of β-adrenoceptors in CRH-induced suppression of antibody responses, rats were administered the nonspecific receptor antagonist propranolol before ICV infusion of CRH or saline and immunization with KLH. To determine the extent to which the adrenal axis mediates CRH immunosuppression, a separate set of experiments was conducted in which the adrenal glands were bilaterally removed from rats before ICV CRH infusion and KLH immunization. The results show that β-adrenoceptor blockade prevented suppression of antigen-specific IgG responses to KLH following CRH infusion. In contrast, adrenalectomy alone reduced the primary antibody response to KLH to such an extent that CRH failed to induce a further suppression of IgM or IgG levels.

MATERIALS AND METHODS

Animals

Male Wistar rats (Charles River Laboratories, Wilmington, MA) weighing an average of 150 g were randomly assigned to treatment groups in the two separate studies:

propranolol or saline ($n = 27$) or adrenalectomy/nonadrenalectomized ($n = 40$). All animals were then housed in pairs in the Animal Research Facility in the Veterans Affairs Medical Center in La Jolla, California for at least 10 days following arrival. The housing environment was maintained at 22°C with lights on at 0600 and off at 1800. Food and water were available *ad libitum.* To reduce exposure to odors or infectious agents from the general animal colony, rats were housed under HEPA-filtered tents (BioClean System, Lab Products, Inc., Maywood, New Jersey). In addition, microisolator filters were placed into the cage lids during transport of the animals for handling and experimental procedures.

Procedures

ICV guide cannula placement. All rats were fitted with ICV guide cannulae approximately two weeks after arrival, at which time animals weighed 250–300 g. Animals were removed from their cages, anesthesia was induced with halothane (1–5%), and permanent indwelling 23-gauge guide cannulae were stereotaxically placed over the lateral cerebral ventricle (coordinates: AP −0.6, ML 2.0, DV −3.2; Paxinos & Watson, 1982) and cemented. Rats recovered for two weeks prior to experimental infusions. To maintain guide cannula patency during this recovery interval, small, sterile, 30-gauge wire stylets (<6 mm in length) were inserted into the cannulae. Manipulation of the stylets was done on a daily basis to habituate the animals to handling and ICV infusion procedures prior to the experimental procedures.

Propranolol treatment. Rats in the propranolol study were randomly assigned to either treatment with propranolol ($n = 14$) or with saline ($n = 13$). Animals were then pretreated with propranolol (20 mg/kg in 0.9% saline IP) or saline 12 h before ICV infusions; propranolol and saline injections were repeated 30 min before ICV CRH or saline. This propranolol dose was previously used by Levine et al. (1988) to demonstrate the involvement of β-adrenergic receptors in joint injury associated with experimental arthritis. Lower doses of propranolol have been shown to block the suppression of NK activity after central CRH infusion (10 mg/kg; Irwin, Hauger, Jones, Provencio, & Britton, 1990) and stress-induced reductions of lymphocyte proliferation responses (2 mg/kg; Cunnick, Lysle, Kucinski, & Rabin, 1990). Two separate propranolol experiments were completed to generate the total number of rats reported for each treatment group.

Adrenalectomy condition. Animals in the adrenalectomy experiments underwent bilateral adrenalectomy or no surgery 10 days prior to experimental infusions under deep halothane anesthesia (5% halothane during induction and 1–2% for the duration of the surgery). Adrenal glands were visually appreciated; adrenal tissue and associated perirenal fat was removed aseptically with forceps. Incisions were closed with wound clips (C. Rivier, consultant). Following surgery, adrenalectomized animals were provided with *ad lib* access to 3% saline in addition to tap water. To verify the efficacy of the adrenalectomy, animals underwent a tail bleed procedure involving brief restraint as described below and blood sampling for assay of plasma levels of corticosterone. Plasma samples were obtained twice: 6 days and 24 h before experimental infusion. Of the animals that underwent adrenalectomy surgery ($n = 32$), 15 were found to have circulating corticosterone levels at or below or 2.2 ng/ml (mean $\pm$ *SD;* 0.2 $\pm$ 0.8 ng/ml) and were assigned to the ''complete adrenalectomy'' condition. The remaining 17 rats had corticosterone levels in excess of 2.2 ng/ml (mean $\pm$ *SD;* 34.2 $\pm$ 28.5 ng/ml) and these animals were considered to have undergone

an incomplete or ''partial adrenalectomy.'' Although visual inspection at the time of surgery indicated complete adrenalectomy in the ''partial adrenalectomy'' animals, it is apparent from the continued detection of low concentrations of corticosterone that these animals experienced some residual adrenal function. It is not known what factors accounted for the high rate of partial adrenalectomy; in some cases the presence of extensive perirenal fat obscured surgical presentation of the adrenal glands. Eight rats served as ''nonadrenalectomized'' controls and had significantly higher levels of circulating corticosterone compared to the two adrenalectomy groups (mean $\pm$ *SD;* 221.4 $\pm$ 183.8 ng/ml; $F(2,40) = 32.4$, $p < .001$). Post hoc comparisons demonstrated that the nonadrenalectomized control animals had higher levels of corticosterone than those found in both adrenalectomy groups ($p < .01$) and that the ''partial adrenalectomy'' animals had higher levels of corticosterone than the ''complete adrenalectomy'' rats ($p < .01$).

ICV infusions. All rats were habituated to human contact for 10 days before experimental infusions by weighing the animals and then handling them for a 5-min period daily. For ICV infusions, the infusion needle (30 gauge) was extended 1 mm beyond the tip of the guide cannula into the lateral ventricle, and CRH (200 pmol; J. Rivier, Peptide Biology Laboratory, The Salk Institute, La Jolla, CA) dissolved in 0.9% saline was infused by gravity during a 30-s period. This CRH dose was selected on the basis of previous experiments in our laboratory in which ICV CRH at this dose produced marked reductions in *in vivo* specific antibody responses to immunization with KLH (Irwin, 1993). Although this dose is higher than levels typically achieved locally during stress (Pich et al., 1995), the final concentration of CRH that ultimately reaches local CRH receptors after ICV infusion is thought to approximate physiological levels. Intracerebroventricular infusion with saline was used in the control animals; previous experiments in our laboratory have determined that ICV infusion of saline does not alter cellular immune responses in handling-habituated animals (Irwin, Hauger, Jones, Provencio, & Britton, 1990). After infusion, the infusion needle was maintained in place for 1 min, the stylet was replaced, and the rat was returned to its cage. Only those rats in which cannular flow was readily observed were used.

Immunization and blood sampling. The soluble protein antigen KLH was used to induce an *in vivo* antibody response. All rats were immunized IP with a 3 μg/kg dose of KLH (Calbiochem, La Jolla, CA) in sterile 0.9% saline. This dose is considered a physiological level of antigen exposure, and it has previously been used in our laboratory to induce robust anti-KLH IgM and IgG antibody responses (Irwin. 1993). Intraperitoneal injection of KLH was performed 20 min after ICV infusion of CRH or saline consistent with our previous procedures (Irwin, 1993).

Blood samples for titration of antibody levels were obtained 1 day before and 3, 5, 7, 9, 11, and 14 days after immunization with KLH using a simple tail venipuncture method for bleeding the unanesthetized rat (Omaye, Skala, Gretz, Schaus, & Wade, 1987). Briefly, rats were restrained, a tourniquet was passed around the tail, and a 20-gauge needle was inserted into a vessel in the midventral surface of the tail. Blood (0.3 ml) was collected using heparinized capillary tubes and spun in a microcentrifuge for 5 min to obtain serum. Aliquots of serum were diluted 1:10 with 0.05% Tween-20 in PBS and stored at $-20°C$ for enzyme-linked immunosorbent assay (ELISA) within 3 months.

Assays

ELISA of KLH antibody. In order to measure KLH-specific IgM and IgG titers in serum, ELISA plates (Maxisorp Immunoplates, Nunc, Copenhagen, Denmark; 96-well flat bottom) were coated with uniform amounts (2.0 μg/well) of KLH in 1 M carbonate saline buffer (Sigma, St. Louis, MO) and incubated for 18 h at 37°C. The coating mixture was removed and wells were filled with blocking buffer (PBS containing 0.1% BSA and 0.02% sodium azide). After a 1-h incubation at 4°C, the blocking buffer was removed and the plates were washed three times with PBS containing 0.05% Tween-20 and 0.02% sodium azide.

Serum samples from the experimental animals were thawed, diluted (1:25, 1:75, and 1:225) in PBS with 0.1% BSA, 0.05% Tween-20, and 0.02% sodium azide, plated in triplicate and incubated at 37°C. After a 4-h incubation, plates were again washed three times. Alkaline phosphatase-conjugated goat anti-rat IgM or alkaline phosphatase-conjugated goat anti-rat IgG antibody (1:5000 in Tween-PBS; Accurate Chemical and Scientific Corp., Westbury, NY) was added to the plates for incubation (18 h at 37°C). For the colormetric reaction, p-nitrophenyl phosphate (1.0 mg/ml in carbonate buffer, Sigma) was added to each well, allowed to develop for 1 h at room temperature, and then measured at 405 nm using a Titertek Multiskan MKII automated plate reader (Flows Laboratory, MacLean, VA). In order to control for potential variability in the results from different ELISA plates, all samples from each individual animal were assayed in the same plate. Inter-assay and inter-ELISA plate variation were determined by adding control rat serum pooled from an animal immunized with KLH (3.0 μg/kg, IP) to each plate.

Corticosterone measurement. Plasma corticosterone was measured in all rats from the adrenalectomy experiments using a commercial radioimmunoassay kit purchased from ICN Biochemicals (Irvine, CA).

Statistical Analyses

Data from each experiment were analyzed independently by three-way mixed analysis of variance (ANOVA) with repeated measures. IgM and IgG data were analyzed separately; similar results were obtained for the three dilutions (1:25, 1:75, 1:225) and the 1:25 data are presented. For the propranolol experiments, the within-subjects factor was day of blood sample (7 samples) and the between-subjects factors were ICV infusion (Saline vs CRH) and peripheral injection (Saline vs propranolol). For the adrenalectomy experiments, the within-subjects factor was day of blood sample and the between-subjects factors were infusion (Saline vs CRH) and surgical treatment (3 levels: nonadrenalectomized, partial adrenalectomy, and complete adrenalectomy). The threshold for statistical significance was set at $p < .05$.

RESULTS

CRH Infusion Suppresses KLH-Specific Antibody Responses

Administration of KLH produced robust increases of serum IgM and IgG antibody levels in ICV saline-infused control rats across the 2 weeks following immunization. Consistent with our previous findings (Irwin, 1993), central infusion of CRH resulted in a significant suppression of both IgM and IgG levels as compared to responses in rats infused with ICV saline (Figs. 1 and 2).

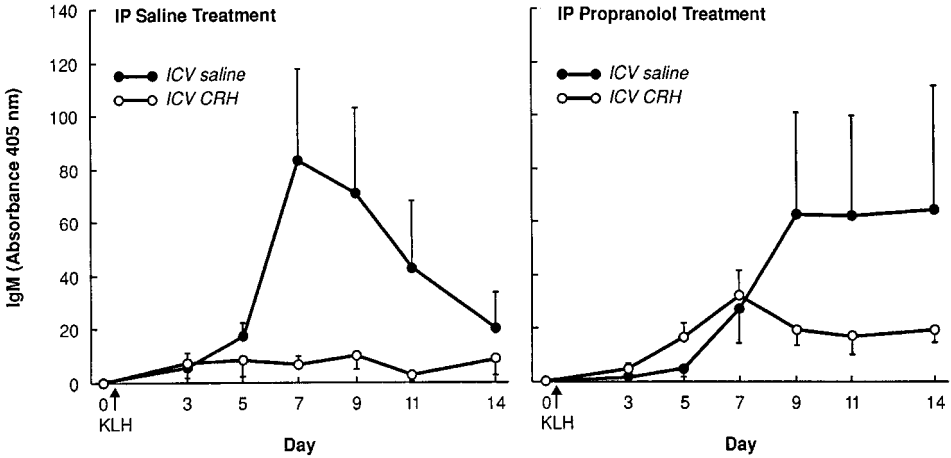

FIG. 1. Antigen-specific IgM antibody titers following IP immunization with KLH (3 μg/kg; units of optical density $\times 10^{-3}$). Data shown are for a 1:25 serum dilution. A 2 (infusion group: saline, CRH) $\times$ 2 (injection group: saline, propranolol) repeated measures ANOVA demonstrated a significant CRH infusion effect [$F(12,276) = 2.2$, $p < .01$], no significant propranolol treatment effect [$F(12,276) = 0.8$, $p = .64$], and no significant infusion by treatment interaction [$F(12,276) = 1.5$, $p = .14$]. Specific contrasts demonstrated significant differences between the CRH and saline groups within both the no injection treatment condition ($p < .001$) and the propranolol injection condition ($p < .001$). Each experimental group contained 6–7 animals.

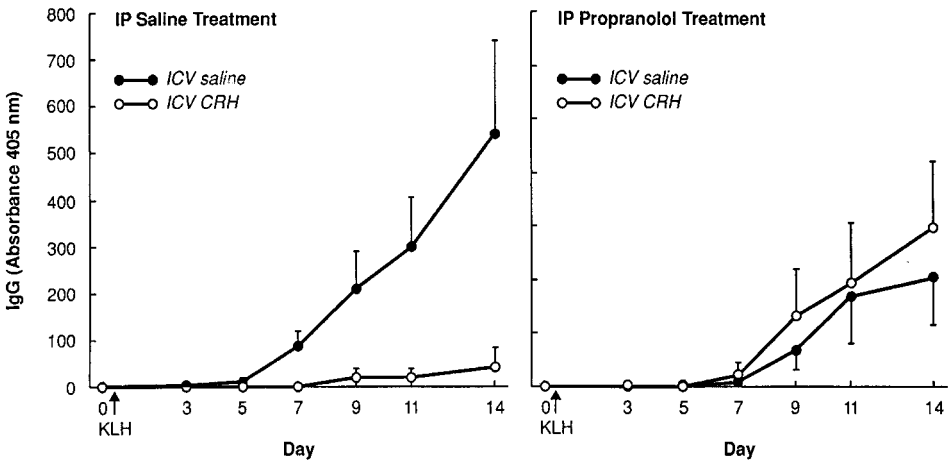

FIG. 2. Antigen-specific IgG antibody titers following IP immunization with KLH (3 μg/kg; units of optical density $\times 10^{-3}$). Data shown are for a 1:25 serum dilution. A 2 (infusion group) $\times$ 2 (injection group) repeated measures ANOVA demonstrated no significant CRH infusion effect [$F(12,276) = 1.6$, $p = .08$], no propranolol treatment effect [$F(12,276) = 0.15$, $p = 1.0$], but a significant CRH infusion by propranolol treatment interaction [$F(12,276) = 2.6$, $p < .01$]. Specific contrasts demonstrated significant differences between the CRH and saline groups within the no injection treatment condition ($p < .001$), but no differences between these two groups within the propranolol injection condition. Each experimental group contained 6–7 animals.

Propranolol Antagonizes CRH-Induced Suppression of Antibody Responses

To evaluate whether β-adrenergic receptor activation mediates CRH-induced suppression of specific IgM and IgG antibody responses, the nonselective receptor antagonist propranolol was given prior to administration of CRH. Pretreatment with propranolol completely abolished the CRH-induced suppression of IgG responses; antibody titers in CRH-treated rats were indistinguishable from those in control animals ($p = .10$; Fig. 2). In contrast, propranolol pretreatment did not reverse CRH-induced suppression of IgM levels (CRH infusion vs saline infusion: $p < .001$; Fig. 1). Propranolol treatment alone had no intrinsic effects on IgM ($p = .15$) or IgG ($p = .16$) responses to KLH immunization compared to rats injected with vehicle, indicating that the kinetics of the IgM and IgG responses in the propranolol treated animals were not different than the controls.

Adrenalectomy Inhibits in Vivo Primary Antibody Responses

Adrenalectomies were performed to assess the role of adrenal hormones in mediating the suppression of primary antibody responses by central infusion of CRH. In the nonadrenalectomized, ICV-saline, control rats, KLH immunization resulted in a significant increase in KLH-specific antibody titers. Central CRH infusion significantly suppressed IgM and IgG specific antibody responses in nonadrenalectomized animals ($p < .01$); (Figs. 3 and 4).

Adrenalectomy that resulted in either a partial or complete removal of the adrenal glands and a significant ($p < .01$) reduction of circulating levels of corticosterone had an intrinsic effect on specific antibody responses. In the partial and complete adrenalectomy groups, KLH failed to induce robust elevations of IgM and IgG levels, and post hoc protected *t* tests indicated that IgM and IgG responses were similarly suppressed in both the partial and complete adrenalectomy groups as compared to nonadrenalectomized control rats (IgM: $p < .05$; IgG: $p < .01$). Central CRH had no additional suppressive effect on KLH antibody responses in either the partial or complete adrenalectomy groups.

To evaluate the relationship between corticosterone and antibody responses in the adrenalectomy groups, a series of correlations between plasma levels of corticosterone and IgM and IgG titers in the total sample and each adrenalectomy group was performed. Plasma corticosterone was only significantly correlated with day 14 IgG levels ($r = 0.55$, $p = .05$) in the total sample. Similar relationships between corticosterone and IgG antibody titers were found in each of the adrenalectomy groups, although statistical significance was not achieved.

DISCUSSION

Consistent with our prior observations (Irwin, 1993), CRH induces a suppression of specific IgM and IgG antibody responses. As central administration of the CRH antagonist α-helical CRH reverses the suppressive effects of central doses of CRH on primary IgM and IgG antibody responses, this effect is argued to be receptor-mediated (Irwin, 1993). The present study tested whether activation of β-adrenergic receptors and/or the pituitary–adrenal axis mediates central CRH-induced suppression of *in vivo* primary antibody responses in rats. Administration of the nonselective β-receptor antagonist propranolol completely abolished the suppressive effects of CRH on IgG levels. In contrast, propranolol pretreatment failed to restore IgM re-

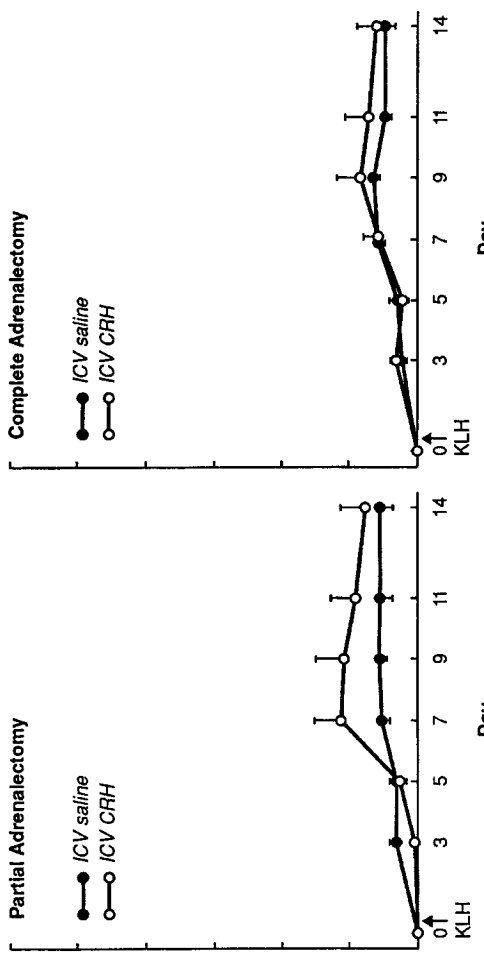

FIG. 3. Effects of adrenalectomy on antigen-specific IgM antibody titers following IP immunization with KLH (3 μg/kg; units of optical density $\times 10^{-3}$). Data shown are for a 1:25 serum dilution. A 2 (infusion group: saline vehicle, CRH) $\times$ 3 (surgery group: no treatment, partial adrenalectomy, complete adrenalectomy) repeated measures ANOVA revealed a significant infusion effect [$F(6,204) = 3.10$, $p < .01$], a significant adrenalectomy effect [$F(2,34) = 4.48$, $p < .05$], and no significant infusion by adrenalectomy interaction [$F(2, 34) = 2.70$, $p = .08$]. Specific contrasts between CRH and vehicle infusions indicated significant differences within the no treatment surgery condition ($p < .05$), but no differences within the partial or complete adrenalectomy conditions. Each infusion group in the no treatment condition had 4 animals and there were 7–10 animals in each of the partial and complete adrenalectomy experimental groups.

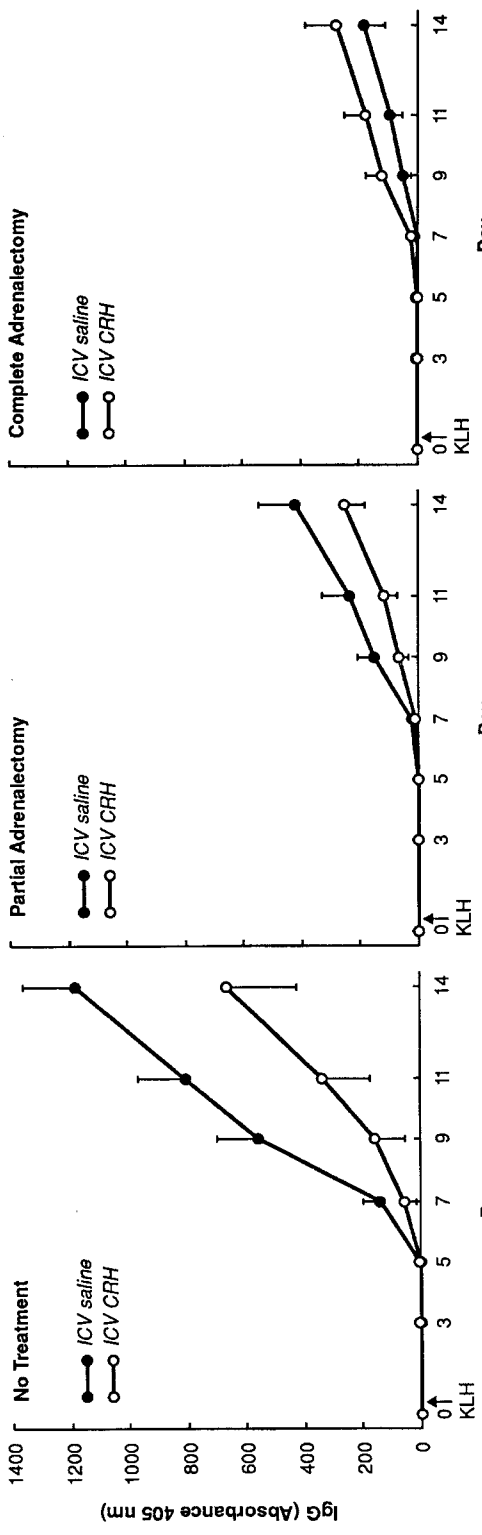

FIG. 4. Effects of adrenalectomy on antigen-specific IgG antibody titers following IP immunization with KLH (3 μg/kg; units of optical density $\times 10^{-3}$). Data shown are for a 1:25 serum dilution. A 2 (infusion group: saline vehicle, 1.0 mg CRH) $\times$ 3 (surgery group: no treatment, partial adrenalectomy, complete adrenalectomy) repeated measures ANOVA revealed a significant infusion effect [$F(6,204) = 2.90$, $p < .01$], a significant adrenalectomy effect [$F(2,34) = 9.30$, $p < .001$], and no significant infusion by surgery interaction [$F(2,34) = 2.90$, $p = .07$]. Specific contrasts between CRH and vehicle infusions indicated significant differences within the no treatment surgery condition ($p < .001$), but no differences within the partial or complete adrenalectomy conditions. Each infusion group in the no treatment condition had 4 animals and there were 7–10 animals in each of the partial and complete adrenalectomy experimental groups.

sponses. Additional studies using different adrenoceptor antagonists across a full dose response range are needed to fully characterize CRH- and stress-induced sympathetic modulation of antibody responses. Nevertheless, the present *in vivo* findings are consistent with *in vitro* observations of Sanders and colleagues that β-adrenoceptor activation modulates primary immune responses (Sanders & Powell-Oliver, 1992), and they extend previous literature on inhibitory effects of CRH-induced endogenous release of sympathetic catecholamines on NK activity and lymphocyte proliferation (Cunnick, Lysle, Kucinski, & Rabin, 1990; Heilig, Irwin, Grewal, & Sercarz, 1993; Irwin, Hauger, Jones, Provencio, & Britton, 1990; Irwin, Hauger, Brown, & Britton, 1988). These data are also consistent with the tonic sympathetic inhibition of humoral immunity reported by Kruszewska et al. (1995) and suggest that *in vivo* release of sympathetic catecholamines with β-adrenergic receptor activation mediates a suppression of specific IgG antibody responses induced by CRH in the brain.

The immunologic mechanisms that account for CRH suppression of IgG responses via β-adrenergic receptor activation remain unexplored, although the T-cell dependent nature of KLH as well as the observed dissociation between IgM and IgG responses in this study both implicate alterations in T-cell regulation of antibody production by B cells. T-cell derived cytokines such as IL-4 and IL-10 stimulate B cell production of IgG1 and IgG3 isotypes (Briere, Servet-Delprat, Bridon, Saint-Remy, & Banchereau, 1994; Lin & Chen, 1993), whereas IL-2 and interferon drive IgG2a production (Lin & Chen, 1993). The current data suggest that β-adrenoceptor activation may be associated with changes in these regulatory cytokines. Indeed, chemical sympathectomy in mice increases antigen-stimulated production of IL-2 and IL-4 *in vitro* (Kruszewska et al., 1995). Because competitive binding studies have further revealed that Th1 clones and activated Th1 cells express β-adrenergic receptors while Th2 clones and cells do not (Sanders, Baker, Ramer-Quinn, Kasprowicz, Fuchs, & Street, 1997; Ramer-Quinn, Baker, & Sanders, 1997), alterations of the Th1 cell and its production of cytokines specifically may underlie changes in specific IgG production that occur following CRH and *in vivo* sympathetic activation. Finally, adrenergic activation of monocyte populations also requires consideration in that Heilig et al. (1993) found that sympathetic activation induced a defect in monocyte function but not in T cells which resulted in diminished antigen specific IL-2 production *in vitro.*

The present results did not support a prominent mediating role for adrenal hormones in CRH-induced suppression of antibody responses. Interestingly, however, partial and complete adrenalectomy resulted in a significant reduction of specific IgM and IgG antibody responses to KLH as compared to nonadrenalectomized controls. To our knowledge, this association between adrenalectomy and impairments in primary antibody responses has not been reported, although others have shown that hypophysectomy impairs multiple aspects of cellular and humoral immunity (Nagy & Berczi, 1978; Keller, Schleifer, Liotta, Bond, Farhoody, & Stein, 1988). The most parsimonious explanation for this observation is nonspecific effects of adrenalectomy surgery on immune function. However, previous studies have found that adrenalectomized animals do not differ from sham-operated or surgically naïve control animals in the expression of MHC class II molecules on peritoneal macrophages (Zwilling, Brown, Feng, Sheridan, & Pearl, 1993), numbers of immunoregulatory cells in circulation (Dhabhar, Miller, McEwen, & Spencer, 1995), or lymphocyte proliferation responses at rest (Keller, Weiss, Schleifer, Miller, & Stein, 1983). Moreover, all rats

in the present experiments underwent ICV cannulation two weeks before the experiments, and we have previously found that surgical placement of ICV cannulae, a procedure involving a longer duration of anesthesia than adrenalectomy, is not associated with immune changes.

Circulating levels of corticosterone in both adrenalectomy groups were lower than those in controls and corticosterone levels were correlated with IgG titers at the 14 day time point in the whole sample. It is possible, therefore, that a threshold, physiological level of corticosterone may be required to facilitate an optimal immunologic response. This hypothesized influence of corticosterone contrasts with its reported immunosuppressive effects during stress, when levels are much higher (Fleshner et al., 1996). The correlation between corticosterone and specific IgG levels suggests further that hormone replacement might have restored the antibody response. Brinkman & Kristofic (1995) have found, for example, that glucocorticoids drive Th cells toward a Th2-like cytokine response that favors humoral immunity.

Loss of adrenal medullary epinephrine would also have occurred following adrenalectomy, and while acute β-adrenoceptor activation may have inhibitory effects, sustained loss and/or decrements of epinephrine may lead to a reduction of antibody responses. *In vitro* data, for example, show that β-adrenergic stimulation of B lymphocytes facilitates the development and expression of specific antibody secreting cells (Sanders & Powell-Oliver, 1992). In addition, adrenalectomy is known to induce increases in central levels of CRH (Owens & Nemeroff, 1991), which may in turn suppress antibody production via elevation of sympathetic tone. Thus, administration of sympathetic blockade to adrenalectomized animals might actually restore or partially normalize antibody titers. Finally, circulating levels of ACTH increase dramatically after adrenalectomy (Zwilling, 1993). ACTH receptors are expressed on mature lymphocytes (Clarke & Bost, 1989) and ACTH has been shown to modulate antibody production *in vitro* (Johnson, Smith, Torres, & Blalock, 1982; Bost, Clarke, Xu, Kiyono, McGhee, & Pascual, 1990), although the influence can be suppressive or stimulatory depending on the dose. Direct influence of ACTH on KLH-specific lymphocytes may thus have contributed to the suppression of IgM and IgG production in the adrenalectomized animals.

In summary, these results further implicate the sympathetic nervous system in the *in vivo* regulation of immune responses. Activation of β-adrenergic receptors by the exogenous administration of CRH or possibly its endogenous release by stress are associated with a reduction of natural immunity and antibody specific immune responses. Moreover, it appears that the adrenal glands play an important role in the primary antibody response to KLH, either through the release of medullary hormones that promote immune function or through the maintenance of an internal milieu that supports optimal immune responses.

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