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Antidepressants reverse the olfactory bulbectomy-induced decreases in splenic peripheral-type benzodiazepine receptors in rats

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Abstract

The present study investigated the effects of 21-day administration of clorgyline (1 mg/kg/day), desipramine (10 mg/kg/day) or paroxetine (10 mg/kg/day) on peripheral-type benzodiazepine receptors in rat peripheral tissues following bilateral olfactory bulbectomy. Thymus and spleen weights decreased as a result of bulbectomy. Subsequent antidepressant drug administration had no further effects on the weights of thymus glands but increased those of spleens. In thymus glands, higher densities of peripheral-type benzodiazepine receptors were observed in medulla than in cortex; no significant variations were observed following bulbectomy or antidepressant drug administration. In spleen, higher densities were observed in white pulp than in red pulp. The bulbectomy-induced decreases in binding densities observed in both regions were reversed following administration of antidepressants. Adrenal peripheral-type benzodiazepine receptors were not altered by bulbectomy or subsequent treatment with clorgyline or desipramine while paroxetine upregulated these receptors. No changes in kidney peripheral-type benzodiazepine receptors were observed. The present study confirms that cell lines of the rat immune system possess high densities of peripheral-type benzodiazepine receptor binding sites and further support the contention that, following olfactory bulbectomy, rats may present an antidepressant-reversible immunitary dysfunction.

Keywords: Benzodiazepine receptor, peripheral type; Olfactory bulbectomy; Thymus; Spleen; Adrenal; Antidepressant

1. Introduction

The induction of depressive-like symptoms in animals has been used extensively in the study of the putative neurological events involved in clinical depression. That, in the rat, olfactory bulbectomy may be a relevant animal model of depression has been suggested on the basis of a large body of experimental evidence. Following bulbectomy, rats display a wide range of antidepressant-reversible behavioural, biochemical and immunological changes, which, in many respects, resemble those observed in patients presenting with major depression (Jesberger and Richardson, 1985, 1986; Leonard and Tuite, 1981; Van Riezen and Leonard, 1990). Several lines of evidence suggest that dysfunction of the hypothalamic-pituitary-adrenal axis may be associated with decreased immune responses. For instance, hypothalamic lesions result in decreases in natural killer cell activity and spleen cell numbers (Mori et al., 1993; Stein et al., 1976). Moreover, corticosteroids suppress T- and B-lymphocytes and natural killer cell activity via interactions with the hypothalamus and the pituitary or through direct effects on lymphocyte corticosteroid receptors (Blalock et al., 1985). It was recently shown that olfactory bulbectomy is associated with suppression of neutrophil phagocytosis, mitogen-stimulated lymphocyte proliferation and plaque-forming cell responses (Komori et al., 1991; Song and Leonard, 1994; Van Riezen and Leonard, 1990). Although similar decreases in the above-mentioned immunological parameters have been reported in depressed patients, some contradictory reports exist (Marraziti et al., 1992; Schleifer et al., 1989; Van Riezen and Leonard, 1990).

Peripheral-type or mitochondrial benzodiazepine receptors have been identified in a wide range of tissues in many species including rats and humans (Benavides et al., 1988, 1989; Drugan and Holmes, 1991). Recent

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evidence suggests that peripheral-type benzodiazepine receptors may be implicated in steroid biosynthesis in adrenocortical cells, associated with anion transport in kidney and may be involved in the early immune response to tissue damage (Drugan and Holmes, 1991; Mukhin et al., 1989; Papadopoulos, 1993). Thus, selective ligands such as PK-11195 (1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline carboxamide) and Ro 5-4864 (4-chlorodiazepam) display immunomodulatory activity. For example, chemotactic activity of human monocytes is enhanced (Ruff et al., 1985) while concanavalin A-induced proliferation of mononuclear cells may be suppressed when high ligand concentrations are used (Bessler et al., 1992). In mice, these ligands produce an enhancement of the T-cell-dependent antibody response towards sheep red blood cells (Lenfant et al., 1986; Zavala et al., 1985). The in vitro and in vivo immunomodulatory effects of peripheral-type benzodiazepine ligands are consistent with the high densities of these sites on the cells of the T- and monocytic lineages in primary and secondary immune system organs of the rat and in lesioned post-mortem human brain tissues, as previously demonstrated (Benavides et al., 1988, 1989).

In light of the reported immunitary dysfunction in bulbectomised rats, in the present study, we investigated the effects of olfactory bulbectomy and subsequent prolonged administration of three classes of antidepressant drugs on [^{3}H]PK-11195 binding to peripheral-type benzodiazepine receptors in peripheral tissues using membrane preparations and quantitative autoradiography. Some of our observations were previously published in abstract form (Dennis et al., 1993).

2. Materials and methods

2.1. Animals, drugs and chemicals

Male Sprague-Dawley rats (COBS-CD, 230–250 g) were obtained from Charles River Canada (St-Constant, Quebec, Canada). Before and after surgery, animals were housed in groups of four and maintained on a 12:12 h light:dark cycle (lights on at 07:00 h) in a temperature- and humidity-controlled room, with free access to standard laboratory chow and water. Animals were weighed at 2- or 3-day intervals over the experimental period.

Drugs and chemicals were obtained from the following sources: [^{3}H]PK-11195 (86 Ci/mmol) from DuPont Canada (Mississauga, Ontario, Canada), clorgyline and desipramine from Research Biochemicals International (Natick, MA, USA), all other analytical grade reagents were obtained from Fisher Scientific (Montreal, Quebec, Canada). Paroxetine was kindly supplied by SmithKline Beecham (Harlow, Essex, UK) and unlabelled PK-11195 was a kind gift from Dr. J.C. Blanchard (Rhône-Poulenc, Vitry-sur-Seine, France).

2.2. Surgery and antidepressant treatments

Olfactory bulbectomy was carried out as previously described (Jesberger and Richardson, 1986). Rats were anaesthetized (chloral hydrate 400 mg/kg i.p.) and mounted in a stereotaxic frame (Kopf instruments). Following a skin incision, bilateral burr holes were made in the skull surface 5 mm anterior to bregma. The dura was pierced, the olfactory bulbs were sectioned and aspirated; the remaining cavities were filled with haemostatic sponge. The incision was sprayed with antibiotic spray and closed with wound clips. Sham-operated rats were similarly operated but the bulbs were left intact.

Antidepressant drug administration began 7 days after olfactory bulbectomy. All drugs were delivered via subcutaneously implanted osmotic minipumps (Alzet, Palo Alto, CA, USA). Rats received either clorgyline (1 mg/kg/day), paroxetine (10 mg/kg/day), desipramine (10 mg/kg/day) or isotonic saline (doses refer to free base of drug). Minipumps were implanted in the scapular region under light anaesthesia with 4% halothane in a nitrous oxide/oxygen mixture (2:1 v/v). The minipumps were removed after 21 days of treatment using the same aneasthesia procedure. Animals were killed by decapitation following a 24 h drug-free period. Brains were quickly removed and inspected macroscopically, all tissues were discarded if any residual tissue of the main olfactory bulbs remained or if the frontal cortex had been damaged during the surgical procedure. Spleens, kidneys, adrenal and thymus glands were rapidly removed, immediately frozen on dry ice and stored at −80°C.

2.3. Membrane binding procedures

All samples removed from individual rats were weighed and assayed separately. Adrenal and kidney tissues were homogenised in 50 vols. of ice-cold Tris-HCl buffer (50 mM, pH 7.4), using an Omni homogenizer (Omni International, Waterbury, CT, USA), setting 7, for 20 s. Following centrifugation at 49000 × *g* for 15 min, the pellet was washed by resuspension and centrifugation as above. Binding assays were carried out in duplicate samples as previously described by Gavish et al. (1987), with slight modifications. [^{3}H]PK-11195 (final concentrations ranging from 0.1 to 10 nM) was added to assay tubes containing 400 μl aliquots of membrane preparations in the presence or absence of unlabelled 1 μM PK-11195 to define non-specific binding. The final assay volume, containing 2–5 or 10–20 μg of protein for adrenal and kidney preparations, respectively, was 0.5 ml. The tubes were incubated for

1 h at 4°C, the assays were terminated by rapid filtration under reduced pressure through Schleicher and Schuell No. 34 glass fibre filters, previously soaked in 0.5% polyethyleneimine. The tubes and filters were washed 3 times with 5 ml of ice-cold buffer and dried. The radioactivity contained on individual filters was determined by liquid scintillation spectrometry.

2.4. Quantitative autoradiographic procedures

For autoradiography, frozen thymus and spleen tissue blocks were mounted on microtome chucks with embedding matrix and allowed to thermoequilibrate in a cryostat at −20°C. Serial tissue sections (14 μm) were cut with a Lipshaw microtome, thaw-mounted onto gelatin-chrome alum-coated microscope slides, rapidly air dried at room temperature then vacuum dried in a desiccator for at least 1 h at 4°C. The sections were stored in sealed boxes at −80°C until used. Tissue sections were processed for autoradiography as previously described (Benavides et al., 1983). Sections were incubated in Tris-HCl buffer (170 mM; pH 7.4) containing 1 nM [^{3}H]PK-11195 for 30 min at room temperature. Non-specific binding was determined by the incubation of adjacent sections in the presence of 1 μM unlabelled PK-11195 and represented approximately 5% of total binding. Following incubation, slides were washed 2 × 5 min in cold buffer, dipped in cold distilled water and rapidly dried in a stream of cold air. The radiolabelled slides were placed in X-ray cassettes along with plastic microscales, a tritium-sensitive film (Hyperfilm, Amersham Canada) was tightly apposed and exposed for 7 days. Optical density measurements were made using a 486 microcomputer based MCID M1 image analysis system (Imaging Research, St-Catharines, Ontario, Canada). A minimum of 24 optical density measurements were carried out in each region of three tissue sections to obtain binding levels for individual animals.

2.5. Protein determination

Protein content of membrane preparations was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

2.6. Statistics

Saturation binding data were analysed by computerised non-linear regression analysis using the EBDA, Ligand, Kinetic, Lowry software package (Elsevier Biosoft, Cambridge, UK) to determine K_D and B_{max} values. The results represent means ± S.E.M. of data obtained from 7–11 rats per group. Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Duncan's test for multiple comparisons, for which the level of significance was accepted as $P < 0.05$.

3. Results

Over the experimental period studied, the sham-operated rats showed a total weight gain of 183 ± 7 g, i.e. a daily weight gain of 6.5 ± 0.3 g. The mean daily weight gain of the saline-treated bulbectomised group of animals was slightly but significantly reduced (5.0 ± 0.4 g). The prolonged treatment of bulbectomised rats with paroxetine had no effect on the daily weight gain (4.5 ± 0.4 g), whereas the weight gain was decreased both by clorgyline (3.1 ± 0.3 g) and desipramine (3.4 ± 0.3 g) administration.

As seen in Table 1, the mean wet weight of thymus glands from bulbectomised rats was significantly decreased compared to that of sham-operated animals. No change in gland weight was observed as a result of antidepressant drug administration. In a similar manner, the mean wet weight of spleens from bulbectomised rats was significantly reduced (Table 1). Prolonged administration of clorgyline and desipramine

Table 1
Effects of olfactory bulbectomy and subsequent prolonged antidepressant treatments on thymus gland and spleen weights in the rat

Pretreatment	Treatment	Thymus		Spleen	
		Weight (mg)	Corrected (mg)[1]	Weight (mg)	Corrected (mg)[1]
Sham	Saline	503 ± 20	120 ± 6	808 ± 19	186 ± 9
Bulbectomy	Saline	387 ± 33[a]	101 ± 8	646 ± 26[a]	162 ± 6
Bulbectomy	Clorgyline (1)	363 ± 32[a]	100 ± 8	685 ± 38	200 ± 8
Bulbectomy	Desipramine (10)	345 ± 46[a]	95 ± 10	717 ± 59	212 ± 19[b]
Bulbectomy	Paroxetine (10)	407 ± 27	106 ± 5	865 ± 69[b]	227 ± 17[b]

Animals were treated with antidepressants (doses in parentheses) for 21 days via subcutaneous osmotic minipumps implanted 7 days after olfactory bulbectomy or sham surgery. Rats were killed following a 24 h drug-free period. Results are means ± S.E.M. of data obtained from 7–11 rats per group. [1]Corrected weight refers to the organ weight in mg relative to 100 g of body weight of animals at death. [a]$P < 0.05$ vs. saline-treated sham-operated animals, [b]$P < 0.05$ vs. saline-treated bulbectomised rats.

was apparently without effect on organ weight, while long-term paroxetine administration reversed the bulbectomy-induced decrease in spleen weight (Table 1). However, measurement of the weight of the organs does not take into account the fact that the treatments reduce the daily gain in total body weight of the animals. Thus, when organ weight is expressed in mg per 100 g of body weight as in Table 1, the 16%

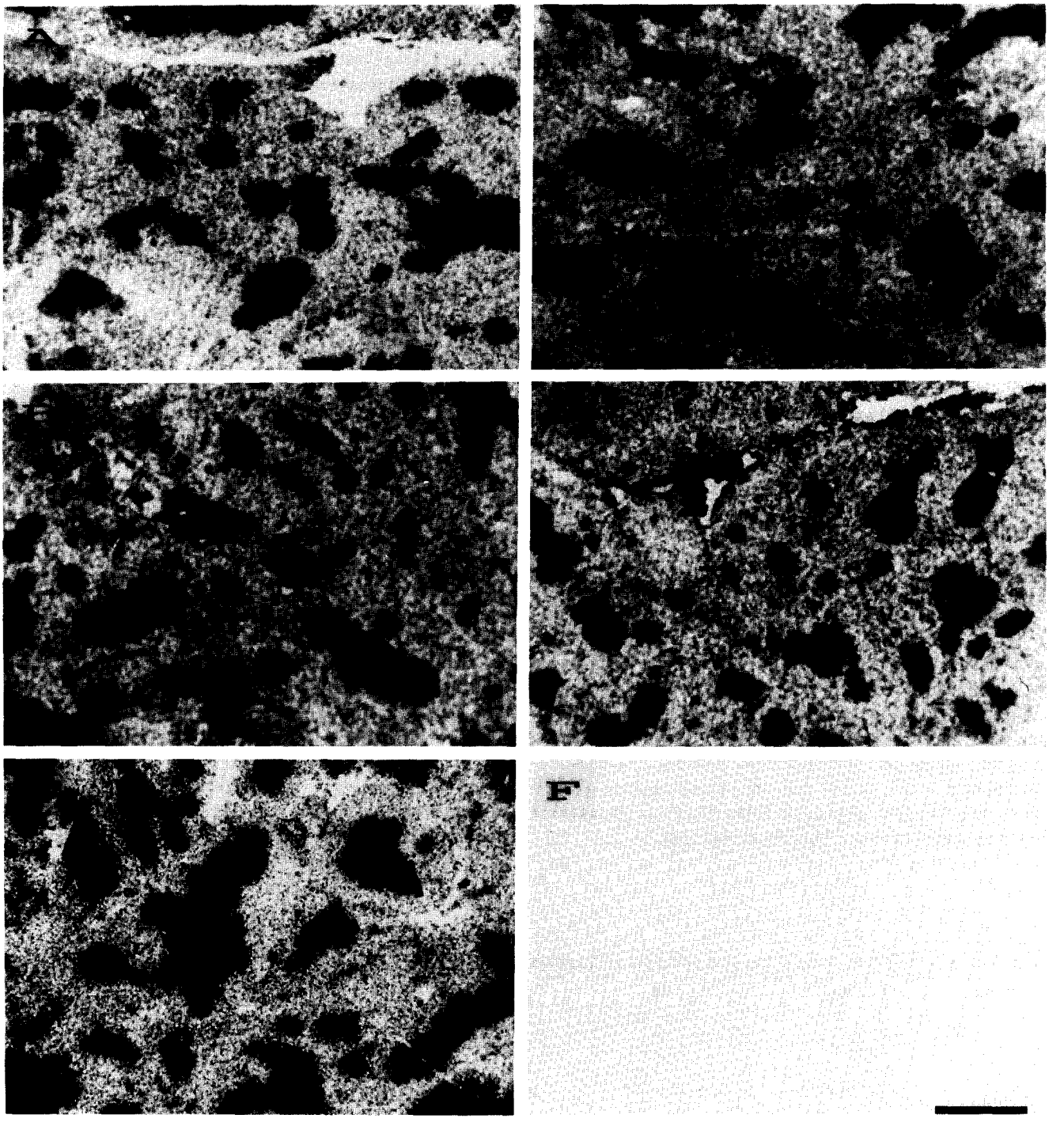

Fig. 1. Brightfield photomicrographs of the autoradiographic distribution of [^{3}H]PK-11195 binding sites in tissue sections of rat thymus gland. Tissue sections were incubated with 1 nM [^{3}H]PK-11195, non-specific binding represented approximately 2–5% of total binding, and was defined by incubation of adjacent tissue sections in the presence of 1 μM unlabelled PK-11195. A: Saline-treated sham-operated rat; B: saline-treated bulbectomised rat; C: clorgyline-treated bulbectomised rat; D: desipramine-treated bulbectomised rat; E: paroxetine-treated bulbectomised rat; F: non-specific binding. Note the higher density of binding sites in the medulla (M) than in the cortex (Cx). Calibration bar represents 1.36 mm.

decrease in relative thymus weight following bulbectomy (compared to 23% in gross wet weight) failed to attain statistical significance ($P = 0.07$), no effects of antidepressant drug administration were observed. Similarly, in spleen, the 13% decrease in relative weight (compared to 20% in wet weight) also approached the limit of statistical significance ($P = 0.08$). This trend was reversed by all three antidepressant drugs, significant increases were observed with desipramine and paroxetine, while clorgyline ($P < 0.07$) failed to attain statistical significance.

As shown in Fig. 1, [^{3}H]PK-11195-labelled peripheral-type benzodiazepine receptors were heterogeneously distributed throughout the thymus tissue. Consistent with a previous report using a photoaffinity ligand (Benavides et al., 1989), quantitative analysis of specific [^{3}H]PK-11195 binding to tissue sections of thymus showed that higher densities of peripheral-type benzodiazepine receptors were found in medullary than in cortical tissues (Table 2). No significant effects of olfactory bulbectomy or subsequent antidepressant drug administration on [^{3}H]PK-11195 binding to peripheral-type benzodiazepine receptors were observed in either region of thymic tissues (Fig. 1, Table 2).

Quantitative autoradiographic analysis of specific [^{3}H]PK-11195 binding in spleen sections showed that peripheral-type benzodiazepine receptors were distributed heterogeneously throughout the red and white pulp of this secondary lymphoid organ (Table 2, Fig. 2). Higher densities of [^{3}H]PK-11195 binding sites were observed in the white pulp than in the red pulp (Table 2). The distinctive distribution of peripheral-type benzodiazepine receptors is shown in Fig. 2, where the highest densities were seen in the marginal zone of the white pulp. Specific binding of [^{3}H]PK-11195 to peripheral-type benzodiazepine receptors was decreased in both red and white pulp one month after olfactory bulbectomy (Fig. 2B, Table 2). Prolonged administration of desipramine (Fig. 2D) and paroxetine (Fig. 2E) completely reversed the bulbectomy-induced decrease in [^{3}H]PK-11195 binding in the red pulp, while long-term administration of clorgyline (Fig. 2C) produced an evident partial reversal which did not attain statistical significance (Table 2). Moreover, desipramine and clorgyline administration reversed and normalised the bulbectomy-induced decrease in [^{3}H]PK-11195 binding in the white pulp, while only a partial reversal was observed with the administration of paroxetine (Table 2).

Saturation analysis of [^{3}H]PK-11195 binding to membranes prepared from whole adrenal glands showed no alteration in binding parameters following olfactory bulbectomy (Fig. 3A). Subsequent treatment of bulbectomised rats with clorgyline produced a slight decrease in B_{max} values which approached the limit of significance. Prolonged administration of desipramine did not alter the B_{max} value for [^{3}H]PK-11195 binding, while chronic paroxetine administration produced a marked increase in B_{max} (Fig. 3A). As seen in Fig. 3B, no alterations of B_{max} values for [^{3}H]PK-11195 binding to kidney membrane preparations were observed as a result of either olfactory bulbectomy or subsequent antidepressant drug administration. No changes in the equilibrium dissociation constant (K_D) values were observed following bulbectomy or antidepressant drug administration either in adrenals (0.69 ± 0.14 nM) or kidney (0.57 ± 0.14 nM).

4. Discussion

Consistent with a previous report using a photoaffinity ligand (Benavides et al., 1989), the results of the present study showed higher densities of [^{3}H]PK-11195-labelled peripheral-type benzodiazepine receptors in the thymic medulla than in the cortex. T cells are generated in the cortex of the thymus from stem cells produced in bone marrow; successive division

Table 2
Quantitative autoradiographic analysis of the effects of olfactory bulbectomy and subsequent prolonged antidepressant treatments on specific [^{3}H]PK-11195 binding to peripheral-type benzodiazepine receptors in thymus gland and spleen of the rat

Pretreatment	Treatment	[^{3}H]PK-11195 bound (fmol/mg)			
		Thymus		Spleen	
		Cortex	Medulla	Red pulp	White pulp
Sham	Saline	247 ± 19	539 ± 28	351 ± 10	579 ± 25
Bulbectomy	Saline	270 ± 16	586 ± 27	273 ± 18[a]	454 ± 20[a]
Bulbectomy	Clorgyline (1)	266 ± 22	551 ± 24	321 ± 25	558 ± 46[b]
Bulbectomy	Desipramine (10)	287 ± 7	611 ± 16	378 ± 10[b]	575 ± 20[b]
Bulbectomy	Paroxetine (10)	266 ± 18	572 ± 34	345 ± 28[b]	528 ± 28

Animals were treated with antidepressants (doses in parentheses) for 21 days via subcutaneous osmotic minipumps implanted 7 days after olfactory bulbectomy or sham surgery. Rats were killed following a 24 h drug-free period. Adjacent tissue sections were incubated with 1 nM [^{3}H]PK-11195 in the absence and presence of 1 μM unlabelled PK-11195 to define specific binding. Non-specific binding represented approximately 2–5% of total and was subtracted from all measured density values. Results are means ± S.E.M. of data obtained from 7–11 rats per group. [a]$P < 0.05$ vs. saline-treated sham-operated animals, [b]$P < 0.05$ vs. saline-treated bulbectomised rats.

during their maturation causes these cells to migrate towards the medulla. This suggests that mature lymphocytes possess higher densities of peripheral-type benzodiazepine receptors in line with their higher state of activation and consonant with the view that these receptors are associated with the outer membrane of mitochondria (Anholt et al., 1986; Benavides et al., 1990; Drugan and Holmes, 1991; Gavish et al., 1992).

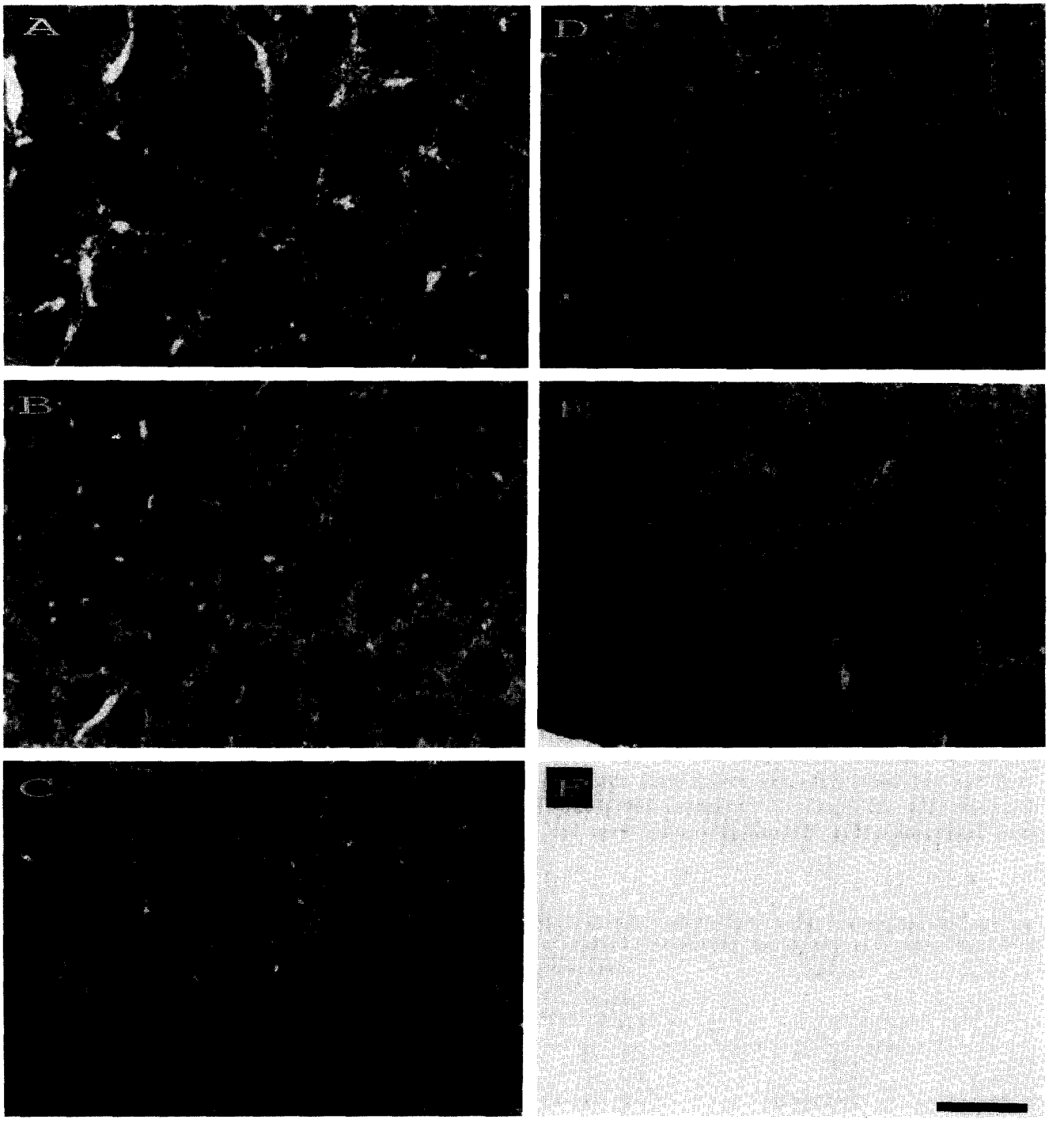

Fig. 2. Brightfield photomicrograph of the autoradiographic distribution of [^{3}H]PK-11195 binding sites in tissue sections of rat spleen. Tissue sections were incubated with 1 nM [^{3}H]PK-11195, non-specific binding represented approximately 2–5% of total binding, and was defined by incubation of adjacent tissue sections in the presence of 1 μM unlabelled PK-11195. A: Saline-treated sham-operated rat; B: saline-treated bulbectomised rat; C: clorgyline-treated bulbectomised rat; D: desipramine-treated bulbectomised rat; E: paroxetine-treated bulbectomised rat; F: non-specific binding. Note the higher density of binding sites in the marginal zone (MZ) of the white pulp (WP) than in the red pulp (RP). Calibration bar represents 1.36 mm.

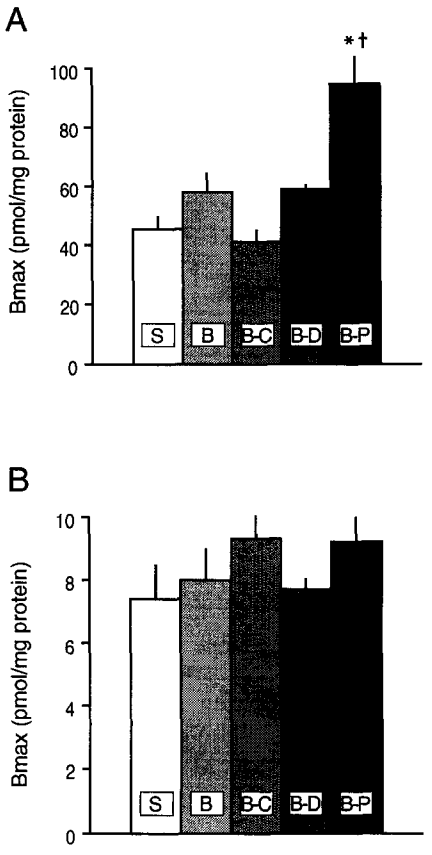

Fig. 3. Effects of prolonged antidepressant treatments on B_{max} values of [^{3}H]PK-11195 binding to peripheral-type benzodiazepine receptors in (A) adrenal gland and (B) kidney of the olfactory bulbectomised rat. Animals were treated with either saline or antidepressants for 21 days via subcutaneous osmotic minipumps implanted 7 days after bulbectomy or sham surgery. Rats were killed following a 24 h washout period. Crude membrane preparations were incubated with six increasing concentrations of radioligand (0.1–10 nM) in the absence and presence of 1 μM unlabelled PK-11195 to define specific binding. Results represent the means ± S.E.M. of data obtained from 7–11 rats per group. S, saline-treated sham-operated rats; B, saline-treated bulbectomised rats; B-C, clorgyline-treated (1 mg/kg/day, 21 days) bulbectomised rats; B-D, desipramine-treated (10 mg/kg/day, 21 days) bulbectomised rats; B-P, paroxetine-treated (10 mg/kg/day, 21 days) bulbectomised rats. $^*P < 0.05$ vs. saline-treated sham-operated animals, $^\dagger P < 0.05$ vs. saline-treated bulbectomised rats.

The highest densities of [^{3}H]PK-11195-labelled peripheral-type benzodiazepine receptors in the spleen were found in the marginal zone of the white pulp. The cells comprising the marginal zone are mostly antigen presenting cells, slowly recirculating B cells, T cells and macrophages. The inner white pulp is composed of periarteriolar lymphatic sheaths which frequently contain germinal centres with mantle zones. The white pulp contains lymphocytes which are free to enter and leave the periarteriolar lymphatic sheaths via capillary branches of the central arteriole in the marginal zone; both T and B cells are found in this region. Some lymphocytes, especially maturing plasmablasts, can pass across the marginal zone via bridges into the red pulp. The red pulp consists of sinuses and cellular cords containing an abundance of phagocytic macrophages, lymphocytes, platelets and plasma cells, making this an active site of antibody production. The existence of peripheral-type benzodiazepine receptors on these different cell types has been documented (Canat et al., 1993).

Olfactory bulbectomy in rodents is associated with a suppression of neutrophil phagocytosis, mitogen-stimulated lymphocyte proliferation and decreased numbers of plaque-forming cells from spleen or bone marrow (Komori et al., 1991; Song and Leonard, 1994; Van Riezen et al., 1990). Moreover, following acute restraint stress, further decreases in the plaque-forming cell populations are observed, contrasting with increases in non-operated and sham-operated controls (Komori et al., 1991). These observations clearly suggest that bulbectomy results in an immunitary dysfunction which may be due to the suppression of immunocompetent cells. Although film autoradiography does not permit resolution at the cellular level, the organ involution and decreases in [^{3}H]PK-11195 binding in the white and red pulp of the spleen observed in bulbectomised rats in the present study support such a notion. However, we cannot rule out the possibility of a decrease in receptor numbers per cell rather than the suppression of immunocompetent cells. On the other hand, this is unlikely as [^{3}H]PK-11195 binding densities in thymic tissues were not altered in spite of the organ involution. Moreover, no changes in peripheral-type benzodiazepine receptor densities were observed in adrenal glands or kidneys (Fig. 3) or in brain areas other than those immediately caudal to the main olfactory bulbs (Beauchemin et al., 1994), i.e. associated with macrophages and activated astrocytes (Benavides et al., 1988, 1990).

The mechanism by which this apparent immunosuppression occurs is at this point unclear. Peripheral-type benzodiazepine receptor densities may be decreased in conditions of chronic stress or anxiety (Drugan et al., 1986; Ferrarese et al., 1990; Weizman et al., 1987), whereas increases in density due to acute stressors such as surgery or forced swim stress are transient and can no longer be detected after 1 week (Novas et al., 1987; Okun et al., 1988). Olfactory bulbectomy may affect the hypothalamic-pituitary-adrenal axis as increased basal plasma corticosterone levels and exaggerated responses to stress have been reported (Cairncross et al., 1977; Jesberger and Richardson, 1986). Thus, it is possible that circulating corticosteroid levels play a direct role via T-cell suppression giving rise to organ involution.

Peripheral-type benzodiazepine binding in adrenals decreases in parallel with their atrophy following hy-

pophysectomy (Anholt et al., 1985) and is restored by administration of adrenocorticotrophic hormone (ACTH) (Fares et al., 1989). Adrenalectomy leads to a decrease in kidney peripheral-type benzodiazepine receptors (Basile et al., 1985), although such an effect was not detected in the aforementioned study (Anholt et al., 1985), whereas chemical sympathectomy with 6-hydroxydopamine was found to have no effect on [^{3}H]Ro 5-4864 binding in various organs including kidney and spleen (Basile and Skolnick, 1988). Thus, just as pituitary hormones like ACTH control adrenal peripheral-type benzodiazepine receptors, adrenal cortex hormones may control renal peripheral-type benzodiazepine receptors.

The recent report of Weizman et al. (1993) showed that 21-day treatments of rats with imipramine or phenelzine both induce a downregulation of peripheral-type benzodiazepine receptors in adrenals but not in kidney. In the present study, we found the monoamine oxidase inhibitor clorgyline, similar to phenelzine, produced a 30% (non-significant) decrease in [^{3}H]PK-11195 B_{max} values compared to the saline-treated bulbectomised rats. On the other hand, we found that prolonged treatment with the tricyclic antidepressant desipramine did not affect [^{3}H]PK-11195 binding parameters. Moreover, long-term administration of the selective serotonin reuptake inhibitor paroxetine induced a 63% upregulation of adrenal peripheral-type benzodiazepine receptors. Long-term administration of the tricyclic antidepressants imipramine and amitriptyline have been shown to attenuate the activity of the hypothalamic-pituitary-adrenal axis. The adaptive changes involve dose- and time-dependent alterations in gene expression of glucocorticoid and mineralocorticoid receptors and corticotrophin-releasing factor leading to decreased secretion of ACTH and corticosterone (Brady et al., 1991; Peiffer et al., 1991; Reul et al., 1993).

There is extensive pharmacological evidence showing that direct- and indirectly acting serotonin agonists activate the hypothalamic-pituitary-adrenal axis via stimulation of the release of corticotrophin-releasing factor (Calogero et al., 1990; Fuller, 1990). On the basis of a number of electrophysiological studies, it has been suggested that a common property of prolonged antidepressant drug administration is the net increase in the efficacy of serotonin transmission (Blier et al., 1990). Thus, tricyclics such as desipramine increase the responsiveness of postsynaptic 5-HT$_{1A}$ receptors (Blier et al., 1990) and amitriptyline and imipramine increase B_{max} values for 5-HT$_{1A}$ receptors in hypothalamus of bulbectomised mice (Gurevich et al., 1993). Clorgyline may desensitise postsynaptic 5-HT$_{1A}$ receptors but the presynaptic effects outweigh the postsynaptic effects (Blier et al., 1990). Paroxetine, like fluoxetine and citalopram (Blier et al., 1990) results in an enhancement of 5-HT neurotransmission following desensitisation of somatodendritic 5-HT$_{1A}$ and terminal 5-HT$_{1B}$ autoreceptors as well as a downregulation of 5-HT transporter sites (Piñeyro et al., 1994). The 5-HT$_{1A}$ receptors involved in the stimulatory effects on the hypothalamic pituitary-adrenal axis may or may not desensitise following prolonged antidepressant treatments (see Chaouloff, 1993). The observed increase in adrenal peripheral-type benzodiazepine receptors following paroxetine treatment indicates increased activity and would argue in favour of a lack of desensitisation.

Clinical studies have shown that intravenous tryptophan administration produces an increase in circulating prolactin levels; this response is blunted in depressed patients and enhanced following a variety of antidepressant treatments including lithium (Price et al., 1989a,b). Prolactin has a positive influence on lymphocyte proliferation; hypoprolactinaemia results in depressed immune responses and elevation of circulating prolactin levels reverses some of the immunosuppressive effects of glucocorticoids and cyclosporin without necessarily reversing tissue atrophy (Ader et al., 1990). The hypoprolactinaemia resulting from olfactory bulbectomy in rats (Pieper et al., 1990) may thus be a contributing factor to their immunosuppressed state.

Given that treatment of bulbectomised rats with desipramine or lithium can, to a large extent, reverse the immunological changes (O'Neill et al., 1987; Song and Leonard, 1994), and the decreases in splenic peripheral benzodiazepine receptors (present study), it would be of great interest to determine prolactin levels in these animals. Despite the blunting of the prolactin response by prolonged clorgyline treatment, bulbectomy-induced decreases in splenic peripheral benzodiazepine receptors were reversed by all three antidepressant drugs tested. This suggests that other, possibly peripheral, mechanisms may be involved. Noradrenergic and peptidergic nerve fibres innervate both thymus and spleen. Stimulation of β-adrenoceptors on thymocytes results in an inhibition of proliferation and enhanced differentiation (Ader et al., 1990). Lymphocytes and monocytes possess β-adrenoceptors and these receptors may be desensitised as the antidepressant treatments used in the present study were shown to downregulate central β-adrenoceptors (Dennis et al., 1994) thereby relieving the restraining effect of norepinephrine on the immune system. Serotonin, on the other hand, exerts immunoenhancing effects and stimulates splenic T-cell proliferation and function (Young et al., 1993).

In conclusion, the results of the present study confirm that cell lines in primary and secondary organs of the rat immune system possess high densities of peripheral-type benzodiazepine receptors. Olfactory bul-

bectomy results in decreased weight of thymus gland and spleen and decreased peripheral-type benzodiazepine receptors in splenic red and white pulp. Long-term antidepressant treatments induced a tissue-specific reversal of the bulbectomy-induced decreases in splenic peripheral-type benzodiazepine receptors and organ weight.

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