ADRENOCORTICAL FUNCTION IN HYPOPHYSECTOMIZED DOGS WITH PAROTID GLAND TRANSPLANTS IN DIRECT CONTACT WITH THE BASAL HYPOTHALAMUS

By

Ramón Alvarez-Buylla and Victor Tsutsumi

ABSTRACT

Adrenocortical activity was studied in three groups of dogs: control-normal, hypophysectomized (hypox), and dogs with a piece of parotid gland grafted into the sella turcica immediately after removal of the whole hypophysis (hypox + graft). Cortisol plasma level ($F_k$) was estimated by the competitive protein binding method. The mean baseline $F_k$ for 21 normal dogs was $0.8 \pm 0.1 \text{ mg/dl}$; for 12 hypox dogs $0.03 \pm 0.01 \text{ mg/dl}$, and for hypox + graft dogs $0.5 \pm 0.2 \text{ mg/dl}$. After mild or severe stresses the normal and hypox + graft dogs showed an increment in $F_k$; hypox dogs showed no change. Adrenal glands of hypox dogs revealed striking diminution in fasciculata and reticularis layers, whereas hypox + graft dogs approached normal. Light microscopy studies of the parotid gland graft showed signs of cellular differentiation. Groups of proliferating cells forming follicular structures were present mainly in the part of the parotid tissue closely associated to third ventricle presenting different staining affinities.

In a previous paper it was shown that in hypophysectomized dogs with a fragment of salivary gland transplanted into the place previously occupied by the pituitary gland (hypox + graft) there was a considerable increase in survival time over that of dogs without any transplant (hypox) (Alvarez-Buylla et al. 1970). Recently, Araujo et al. (1975) also found a longer survival time in parotid grafted dogs after hypophysectomy. Of all the pituitary functions.

1) Dirección General de Investigación Médica de la Subsecretaría de Asistencia de la S.S.A., México, D.F.
adrenal control is probably the most important for this increased survival (Jacobson 1966). The transplant of salivary gland after hypophysectomy has been reported to increase plasma 11-hydroxycorticosteroid level with respect to that found in hypox animals (Alvarez-Buylla & Alvarez-Buylla 1970), as well as to improve \(^{131}\)I uptake by the thyroid (Alvarez-Buylla et al. 1971). In the present communication a comparative study of the adrenocortical function and morphology was done in three groups of dogs: normal, hypox and hypox + graft dogs. Plasma cortisol concentrations (\(F_k\)) were determined in resting conditions, and their changes evaluated after mild and severe stresses. Comparative morphological studies of adrenal cortex of the three groups of dogs were done.

**MATERIAL AND METHODS**

*Animal and surgical procedures.* – Forty-one male and female adult mongrel dogs were used in this investigation. To avoid undue stress to the animals they were pre-housed for at least fifteen days prior to the operation or blood sampling, given water and food *ad libitum* and handled regularly. All through the experiment these conditions were maintained. Polyethylene catheters (Clay Adams PE-90) filled with a solution of heparin (200 U/100 ml saline) were permanently inserted into the saphena vein and blood flow was maintained using a technique previously described (Alvarez-Buylla & Alvarez-Buylla 1975). Blood samples were made through these cannulated vessels. Hypophysectomies and parotid gland auto-grafts into the sella turcica were performed by the temporo-parietal approach according to the Alvarez-Buylla et al. (1973) techniques. To assure a complete hypophysectomy, the pituitary gland was excised cutting in the basal hypothalamus above the pituitary stem; therefore the gland obtained was uninjured and with a piece of infundibulum. The tissue graft taken from the same animal closed the opened third ventricle. All operated dogs were studied at least 2 months after the operation.

*Biochemical methods.* – Venous blood samples were collected in heparinized tubes and immediately centrifuged. One aliquot of the plasma supernatant was used for \(F_k\) assay by the competitive protein binding method (Corker et al. 1971). In some cases glucose assays were performed by the Ortho-Toluidine method (Hultman 1959).

*Experimental design.* – The dogs were divided into three groups chosen at random: (a) control, (b) hypox and (c) hypox + graft. Blood samples were always taken in fasting unanaesthetized animals between 9 and 10 a.m. The animals were subjected to three experimental interventions: (1) basal blood sampling, the mean of eight determinations in each dog made on different days; (2) bolus intravenous (iv) injection of glucose (0.5 g/kg Dextrabbot) by puncturing the vein, as a mild stress; (3) ether inhalation for one minute (min), as a severe stress. The sampling period during mild or severe stresses was 60 min after a basal determination. The values are means of 3 tests in each dog. To provide direct comparison of the response in stress experiments, the \(F_k\) data in Fig. 2 have been expressed as the changes in the concentration from the values at time zero (stress stimulus). Significance of differences was examined with Student's *t*-test; when the variances were not homogeneous the Student's *t*-test modified by Cochran & Cox (1972) was used. Values were considered significant if \(P < 0.05\).
Morphological procedures. — Dogs were anaesthetized with sodium pentobarbital (40 mg/kg, iv) and were sacrificed while being bled through the vena cava by a rapid injection of 10% formalin solution through a cannula placed in the carotid artery; during the time of perfusion the respiratory rate was maintained by an artificial pump. After death the head was opened and a block containing the hypothalamic – hypophyseal zone was removed for fixation, inclusion and frontal sections according to a previously described technique (Costero et al. 1975). Serial sections (7 μm) stained with haematoxylin-eosin were carefully revised looking for adenohypophyseal remnants. Adrenal glands from the three groups of animals were promptly excised. For light microscopy whole adrenal glands were fixed in formalin and processed with the routine method for paraffin-embedding.

![Graph](image.png)

*Fig. 1.*

Basal concentration of cortisol in plasma. Eight determinations were done in each dog. Vertical lines on columns represent sem. In parenthesis number of animals.
RESULTS

Basal cortisol in plasma. – While the animals were maintained in resting conditions, in 21 normal dogs the median basal $F_k$ was $0.8 \pm 0.1 \mu g/dl$, the level in 12 hypox dogs was $0.03 \pm 0.01 \mu g/dl$, in 8 hypox + graft dogs the mean basal $F_k$ was $0.5 \pm 0.2 \mu g/dl$, statistical comparison between hypox dogs and hypox + graft dogs was significant ($P < 0.05$), as was the comparison between normal and hypophysectomized values ($P < 0.01$). (Fig. 1).

Mild stress. – Control animals which received a bolus iv injection of glucose displayed a significant rise in control levels, peaking 30 min after the injection (Fig. 2A). The mean basal $F_k$ in 3 control dogs was $0.8 \pm 2 \mu g/dl$; 30 min after the injection it increased to $3 \pm 1 \mu g/dl$, and returned to $0.5 \pm 0.3 \mu g/dl$ 60 min

Fig. 2.

Changes in cortisol concentration in response to stress. The basal value is considered as zero. The values are means of 3 tests in each dog. Vertical lines represent SEM. In parenthesis number of animals.
Fig. 3.
Fig. 4.
Cortex of adrenal gland. (A) normal dog. (B) six month hypox dog. (C) 24 month hypox + graft dog. The vertical bars represent the thickness of the adrenal cortex. (g) zona glomerulosa. (f) zona fasciculata. (r) zona reticularis. (m) adrenal medulla. × 100.

Fig. 3.
Light microscopy of the parotid gland transplanted area. (A) Dog sacrificed two weeks after the grafting. The part of the transplant closely associated to the third ventricle (HIV) presents group of cells proliferating and forming follicular structure (arrows). (f) fibrous tissue × 40. (B), ten weeks after the operation. There is a glial reaction (g) which has closed the third ventricle. More compact group of cells forming follicles and cords of cells (t) are present × 100. (C) Higher magnification of the ten weeks transplant. Cells with different staining affinities are seen. Amorphous material is present in the follicular lumen (arrows), × 550.
after the injection. The difference between the basal level and the level at 30 min was statistically significant \( (P < 0.01) \). The mean glucose concentrations in these dogs increased from 90 mg/dl to 120 mg/dl after the injection. Five hypox dogs showed no significant change in each point after the injection, glucose increased from 60 mg/dl to 110 mg/dl. In 5 hypox + graft dogs the following results were obtained: 0.2 ± 0.1 \( \mu \)g/dl (basal), and 1.8 ± 0.1 \( \mu \)g/dl (30 min) and 0.5 ± 0.2 \( \mu \)g/dl (60 min) after the injection. The basal level of glucose in transplanted dogs was 70 mg/dl increasing after the injection to 115 mg/dl. If we assign a value of zero to the basal level of \( F_k \) in each group, after a mild stress the normal dogs showed an increment of 2.2 \( \mu \)g/dl; hypox + graft dogs increased their level by 1.6 \( \mu \)g/dl, and hypox dogs showed no increment (Fig. 2A).

**Severe stress.** – Animals which received an ether inhalation for 1 min showed a marked and rapid increase in \( F_k \), peaking at 20 min after the exposure to ether. Six control dogs showed a mean basal \( F_k \) of 2.7 ± 1 \( \mu \)g/dl, 20 min after the stressor stimulus, the level increased up to 10.2 ± 2 \( \mu \)g/dl, and by 60 min the level was 9.2 ± 2.5 \( \mu \)g/dl. The differences between the basal level and the levels at 20 and 60 min were statistically significant \( (P < 0.01) \). In 5 hypox + graft dogs the basal values obtained were 1.4 ± 0.3 \( \mu \)g/dl, 20 min after the ether application increased to 4.2 ± 2 \( \mu \)g/dl and 3.7 ± 1.9 \( \mu \)g/dl at 60 min. Comparing the basal level with the values obtained at 20 and 60 min significant differences were obtained \( (P < 0.05) \). In the same conditions, hypox dogs showed no significant change after ether inhalation (Fig. 2B).

**Light microscopy.** – Two weeks after the parotid tissue grafting, no foreign-body reaction was observed. Groups of proliferating cells forming follicular structures of different sizes (Fig. 3A) were present mainly in the part of the parotid graft closely associated to third ventricle. The part of the graft localized distant to third ventricle displayed a fibrous reaction. Ten weeks after the grafting, the third ventricle opening was closed by a glial tissue reaction (Fig. 3B), although, the ependymal lost during the hypophysectomy was not reinstalled again. Cells from the transplant started to show some signs of differentiation presenting different staining affinities (Fig. 3C). The lumen of the follicular cells presented amorphous material which were PAS (periodic acid Schiff) positive. Fig. 4 shows histological differences of the adrenal cortex of a normal dog (A), a dog 6 months after hypophysectomy (B), and a dog 24 months after hypophysectomy and parotid transplant (C). The hypox dog had a striking diminution in fasciculata and reticularis layers, whereas the glomerular layers seemed unchanged or slightly hypertrophied. Hypox + graft dogs showed adrenals with much lesser degree of alterations than in the hypox dog, and the width of the adrenal cortex was essentially similar to that of the normal dog.
DISCUSSION

In a previous paper (Alvarez-Buylla & Alvarez-Buylla 1970) a reduced survival time was shown in totally hypox dogs. Microscopic examination revealed advanced atrophy of ovaries and testes as well as an important loss of thyroid function. The increased survival time observed in hypox + graft dogs may well be due to the recovery of some pituitary functions, principally the adrenal function. In hypox + graft dogs adrenal histology approached normal (Fig. 4) improving the hypox image where the lack of ACTH is the cause of a profound atrophy of fasciculata and reticularis layers.

The mean F$_k$ values obtained in control dogs were comparable with those obtained by Drost et al. (1973), Harwood & Mason (1976) and McNatty & Young (1973). Much higher basal F$_k$ have been reported in dogs in previous papers (Clements & Newsome 1973; Sadowski et al. 1975). As dogs are readily excited by minor environmental disturbances, and as the F$_k$ rises within 1 min of the release of ACTH into the systemic circulation (Setchell et al. 1975) it would seem very probable that in earlier works the basal F$_k$ reflects some degree of stress. In this study special care was taken to avoid stress and the dogs were adapted to the experimental environment. Most of the previous investigations have been based on fluorometric assays (Rijnberk et al. 1968a,b). A method utilizing the steroid binding properties (CPB) of corticosteroid-binding globulin was described in 1968 for the determination of corticoids in plasma (Murphy et al. 1963); apart from great sensitivity, the advantage of this method is the absence of effects due to non steroid substances. Corker et al. (1971) applied the method further to measure F$_k$ in dog plasma. Cortisol values measured by CPB methods have been lower than those based on fluorometric assays (James et al. 1967; Sarin et al. 1971).

The present experiment demonstrates that two kinds of stresses in normal dogs elicit clear increments in F$_k$ (Fig. 2). In the case of mild stress the puncture of the vein rather than the hyperglycaemic response was the stressor stimulus, since in previous experiments it was shown that the hyperglycaemia without any other stimulus did not produce changes in F$_k$ in dogs (Becerra 1976). The normal and hypox + graft dogs responded to mild stress in almost identical form with increments in F$_k$ at 30 min and recovery at 60 min, being the response to severe stress of greater magnitude for both groups, although more intense in normal than in hypox + graft dogs. In contrast, the hypox dogs showed no change in F$_k$ after either stress type (Fig. 2). The 98 % reduction in basal F$_k$ levels found in hypox dogs could be explained as a lack of ACTH secretion by the pituitary gland. It has been shown earlier that hypox dogs have a very low hydroxycorticosteroid level in adrenal plasma and these values did not rise in response to stress (Eik-Nes & Brizee 1958; Heap et al. 1966). The F$_k$ found in hypox + graft dogs was only 60 % of that of control dogs: these values

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could explain the longer survival time obtained in hypox + graft (Alvarez-Buylla et al. 1970), which is five times longer than that of hypox dogs without any transplant. The same parotid tissue transplanted outside the median eminence region did not induce any change in survival time of hypox dogs (Costero et al. 1975).

In serial sections of the operated area no remnants of adenohypophyseal tissue were found in hypox and hypox + graft dogs studied in this experiment. The microscopic structure of the fragment of transplanted parotid presents during the first month changes in cellular organization, forming follicular or irregular cords of cells closely associated to capillaries. Cellular changes, characterized by the different staining affinities, appear after the first month of grafting. These changes as described in a previous paper (Costero et al. 1975) occur mainly in the part of the parotid fragment intimately associated with the third ventricle suggesting the presence of some hypothalamic factors which may produce these changes on the parotid gland. A detailed study on the time-sequence changes of the transplanted tissue, using the electron microscope and immunohistochemistry will be reported later. Apparently, an important correlation exists between functional behaviour of the hypox + graft dogs and the histological state of the transplanted parotid tissue. Rodríguez & Piezzi (1967) have shown that after removing the pars distalis of a toad, the pars intermedia is penetrated by the portal vessels and, after a few weeks, pars distalis-like cells appear in the region of the pars intermedia irrigated by the portal capillaries. Cuello & Rodriguez (1977) also working in toads did not find pars distalis-like cells in adrenal tissue grafted into the median eminence - pars distalis region, but the tissue became revascularized by the portal vessels. In this work the authors did not report the physiological condition of the animals.

In the adrenal cortex, the glomerulosa layer appears to be little affected, either by hypophysectomy or hypophysectomy + transplant, and the changes observed are of active cells without signs of degeneration. In contrast, fasciculata and reticularis layers are importantly affected in the hypox dogs, where after a few weeks, most of the cells suffer degeneration and the typical lipid droplets lose their opacity, appearing as clear vacuoles, as described by others (Bloodworth 1966). In the hypox + graft dogs, the morphological features of the fasciculata and reticularis layers are those of the glands that partially synthetize hormones, therefore we assume that a hypophyseal substance which stimulates the adrenal gland is probably produced in the transplanted tissue under the effects of a hypothalamic factor.
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REFERENCES


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