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Contents

Long-term survival of autologous adrenal medulla grafts in the great omentum of the rat

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The omentum, a rich source for trophic and angiogenic factors, was explored as a potential intermediate transplant site to facilitate long-term survival of chromaffin tissue. Autologous rat adrenal medullas were grafted into omental pockets. All grafts became densely vascularized. The grafted chromaffin tissue exhibited strong immunoreactivities for tyrosine hydroxylase, synaptophysin and chromogranin A throughout the observation period of 16 weeks. The expression of these markers implies that grafted chromaffin cells retained the key enzyme for catecholamine biosynthesis and the organelles required for catecholamine secretion. Moreover, intermediate transplant of chromaffin tissue to the omentum could provide a favourable conditioning microenvironment thus augmenting the potential for survival of functional chromaffin tissue. [Neurol Res 1993; **15**: 269–272]

Keywords: Great omentum; adrenal medulla; autotransplantation; Parkinson's disease, immunocytochemistry

INTRODUCTION

Despite promising preliminary reports[1,2], clinical and experimental investigations for direct transplantation of autologous adrenal medulla chromaffin tissue into the brain have yielded only short-term amelioration of catecholamine deficiencies[3,4]. Lack of trophic factors and poor or delayed vascularization may have contributed to the rapid cell death of the chromaffin cells.

There are several reasons to believe that the great omentum could serve as an ideal intermediate conditioning site for adrenal medullary grafts. It is a densely vascularized organ and contains a variety of angiogenic and neurotrophic factors[5,6].

Here we report on the long-term survival of adrenal medullary cells when transplanted into the great omentum of the rat. We examined the vascularization of the transplanted tissue and the function of the chromaffin cells as indicated by the expression of tyrosin hydroxylase, synaptophysin and chromogranin A.

METHODS

Twenty-seven adult wistar rats (250 g body weight at the time of implantation) served as both tissue donors and recipients. The left adrenal was removed under anaesthesia (5% chloral hydrate, 1 ml intraperitoneal; 0.5 ml Atropine, 0.1 mg ml^{-1} subcutaneously) and placed in lactated Ringers Solution. The resected cortex of the adrenal gland was microdissected from the medulla and the medulla cut into approximately 1 mm pieces for subsequent transplantation. A small pocket was made between the two membranes of the great omentum near the gastroepiploic artery or its branches. After insertion of medullary tissue pieces, the pocket was closed with two sutures (10-0) and marked with a mini Hemo-Clip.

The autografts were examined one day, 2, 4, 6, 8, 10, 12, 14 and 16 weeks following transplantation. There were 3 rats in each group except the 8 week group which consisted of 2 rats. The excised transplants were immersed in Bouin's fixative for 6 h, subsequently dehydrated with ethanol, embedded in paraffin and sectioned at 4–6 micrometers.

Immunocytochemistry

The avidin–biotin–peroxidase (ABC) technique[7] was performed on deparaffinized paraffin sections. Endogenous peroxidase activity was blocked with 0.3% H_2O_2 and 10% methanol in phosphate-buffered saline (PBS). Sections were made permeable in Triton-X 100 (0.5% in PBS, pH 7.4) for 5 min. Subsequently some sections were incubated (20 min) with 10% normal goat serum prior to incubation with synaptophysin (SYN) – or chromogranin A (CGA) – antiserum. Other sections were incubated (20 min) with 10% normal horse serum prior to incubation with the tyrosine hydroxylase (TH)–antiserum. The sections were then incubated overnight with polyclonal rabbit serum against rat SYN (antibody G95, dilution 1:2000, kindly provided by Dr R. Jahn, New Haven, CT, USA), a rabbit antiserum directed against the C-terminal 16 amino acid sequence of bovine CGA (dilution 1:1000, kindly provided by D.T. O'Connor, San Diego, CA, USA) or with anti-TH (monoclonal, dilution 1:1000, Boehringer Mannheim, Germany).

For the detection of SYN and CGA, biotinylated anti-rabbit IgG (diluted 1:500, Vector, Burlingame, CA, USA) and ABC-complex (diluted 1:200, Vector, Burlingame, CA, USA) were used in the second and third steps of the immunostaining. Biotinylated anti-mouse IgG (diluted

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1:500, Vector, Burlingame, CA, USA) was used as second antibody during immunostaining for TH. The immunoreaction was visualized with a freshly prepared solution of 0.05% 3,3' diaminobenzidine–tetrahydrochloride (Aldrich, Milwaukee, USA) and 0.01% H_2O_2 in 0.1 M Tris–HCl buffer (pH 7.6) for up to 10 min. In control sections the primary antibody was replaced by normal serum. Sections were photographed using a Zeiss-Photomicroscope. Other sections were stained with haematoxylin and eosin.

RESULTS

General

All rats appeared healthy throughout the observation period except for one rat in the 8 week group which developed an abdominal wall herniation and was excluded. Macroscopic observations of the transplants *in situ* revealed neovascularisation in the graft sites beginning two weeks after transplantation (*Figure 1*). At the light microscopic level none of the transplants showed any signs of haemorrhage or chromaffin tissue degeneration. In the conventional haematoxylin and eosin stains we observed abundant capillary networks around the grafts. No obvious evidence of inflammatory reaction was noted.

Immunocytochemistry

As shown in *Figures 2* and *3* chromaffin cells grafted in the omental pocket exhibited strong immunoreaction against characteristic antigens of chromaffin cells. This holds a) for tyrosine hydroxylase, which is the main regulatory enzyme in catecholamine biosynthesis;[8] b) for the intracellular membrane antigen synaptophysin[9–11] and c) for chromogranin A[12,13], which is stored and released together with the catecholamines from adrenal medullary chromaffin cells. Interestingly, subjective observations of control (i.e., one day after transplantation) immunoreactivities were not markedly different from immunoreactivities 2 or 16 weeks after transplantation. The grafted chromaffin cells retained their endocrine phenotype and did not develop processes.

DISCUSSION

Since adrenal medullary cells synthesize dopamine and have the potential for growing neuronal-like processes[14–16], they have been suggested as a possible replacement for the dopamine neurons which are lost in Parkinson-like disease. However, the survival of grafted chromaffin cells in the brain is limited. Studies in several species including humans showed disappointing results concerning viability of chromaffin cells[17–23]. Only few adrenal chromaffin cells survive neural implantation unless supplemented with exogenous NGF[23]. Another strategy to improve survival involves cografts of chromaffin cells with other cells producing trophic factors[24]. Surprisingly even tissue grafts containing no chromaffin cells ameliorated experimental parkinsonism[24–27].

In our approach to enhance the survival of adrenal medullary cells we explored the omentum as an intermediate site for the adrenal medullary grafts. The omentum is a multipotent organ with angiogenic as well as neurotrophic factors[5,28–32] which could provide a vascularized and conditioning microenvironment for the chromaffin cells. Lack of growth factors might be one of the reasons for rapid degeneration and death of chromaffin cells transplanted into the brain. It is well known that the survival of chromaffin cells depends on such factors and in addition themselves produce growth factors[33]. Furthermore – and relevant to transplantation – chromaffin cells are able to grow processes in response to NGF and bFGF[14–16]. Also fibre outgrowth in the host tissue suggests that the adrenal medullary grafts provide important trophic factors for striatal dopaminergic neurons[17]. The great omentum

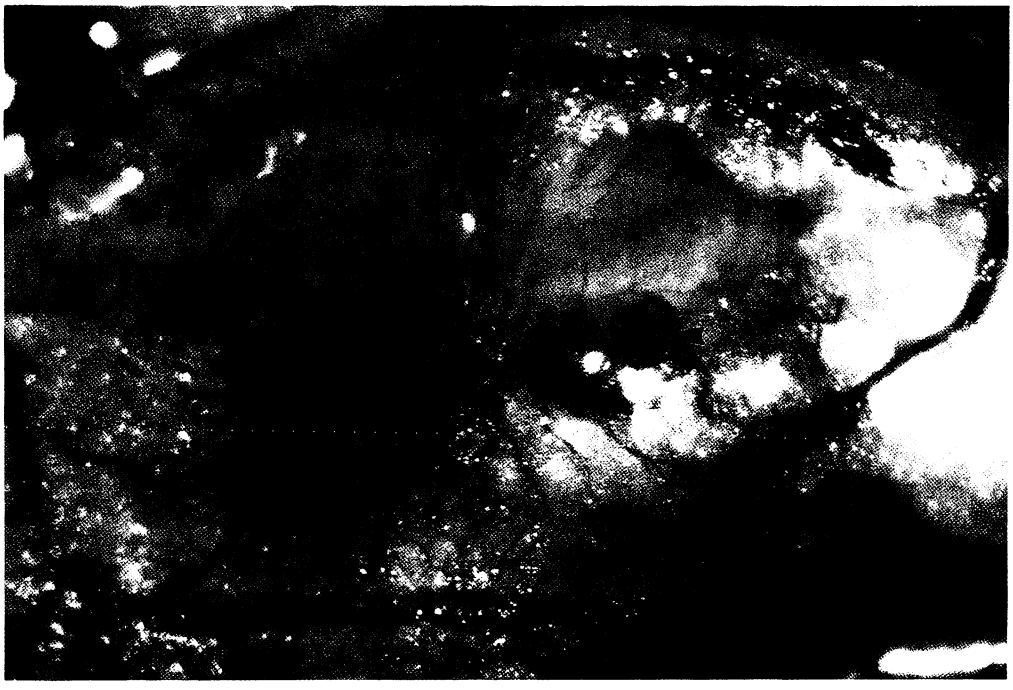

Figure 1: A low magnification view of an adrenal medulla autograft within the omentum, 4 weeks after graft implantation. Neovascularization (▶) builds a network around the transplant (→) × 10

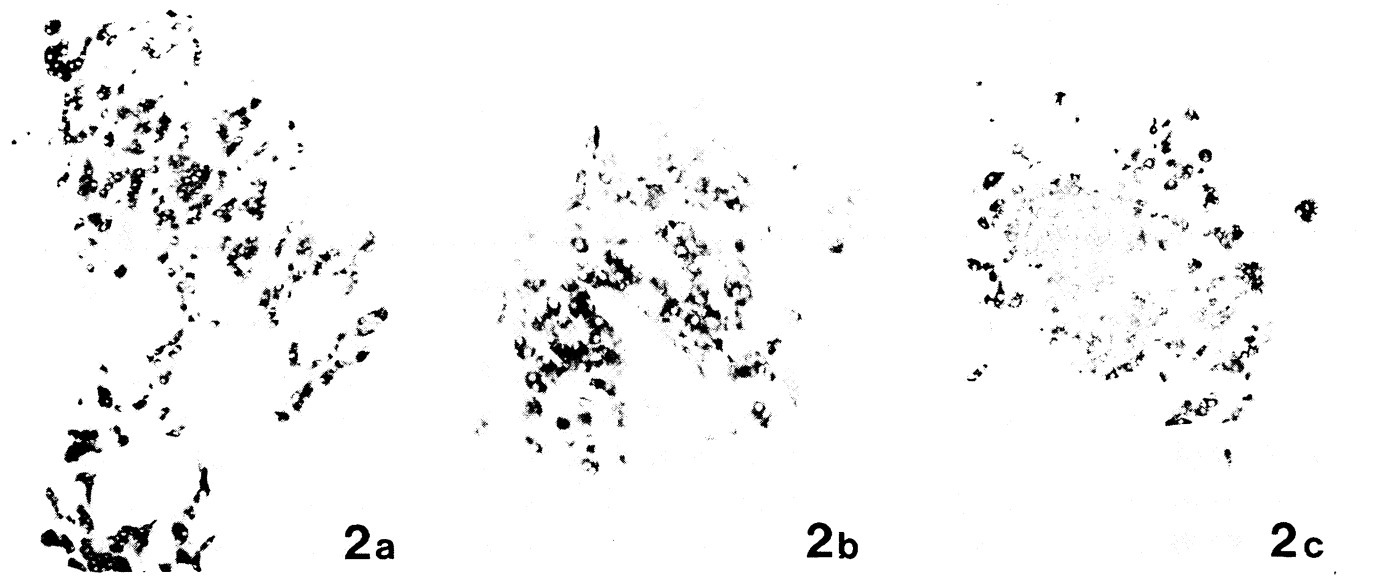

Figure 2: **(a)** Immunocytochemical evidence of graft survival in the great omentum 2 weeks after transplantation. **(b)** The graft shows pronounced TH-immunoreactivity × 20. **(c)** Surviving chromaffin cells demonstrate strong chromogranin A immunoreactivity × 20 and synaptophysin expression × 20

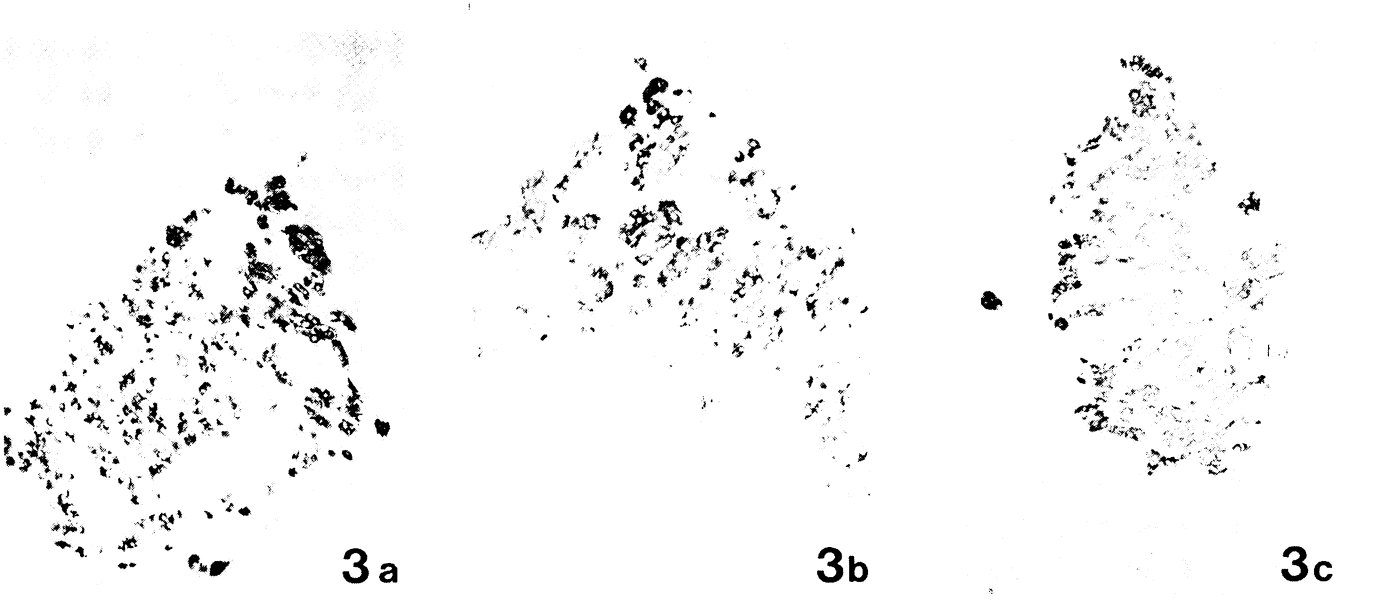

Figure 3: Immunocytochemical evidence of graft survival in the omentum 16 weeks after transplantation, a strong immunoreaction in the grafted adrenal medulla is still evident. **(a)** Tyrosine hydroxylase × 20. **(b)** Chromogranin A × 20. **(c)** Synaptophysin × 20

is a highly vascularized organ producing a variety of angiogenic and trophic factors which promote survival of transplanted chromaffin cells. Acidic FGF and basic FGF have been purified from bovine omentum[32]. Basic FGF has also been isolated from human omentum and high and low affinity receptors for basic FGF have been demonstrated in human omental microvascular endothelial cells[5].

Our results demonstrate long-term survival of autologous adrenal medulla grafts in the rat omentum (16 weeks). It is reasonable to assume that surviving chromaffin cells are functionally intact because of their strong tyrosine hydroxylase, synaptophysin- and chromogranin A immunoreactivities.

In conclusion, our results indicate that adrenal medulla grafts in the rat omentum have a long-term survival potential. In the next step we will examine if this tissue survives in its subsequent CNS transplantation and is immunocytochemically viable.

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