Transmitter expression and morphological development of embryonic medullary and mesencephalic raphé neurones after transplantation to the adult rat central nervous system

I. Grafts to the spinal cord

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Summary. Suspensions of cells derived from the mesencephalic raphé or medullary raphé regions of the 13–14 day old embryonic rat brain were injected into the spinal cord of adult rats which had been previously denervated with 5,7-dihydroxytryptamine. At periods of up to 12 months after grafting, the spinal cords were taken for immunohistochemical analysis of 5-hydroxytryptamine (5HT), substance P (SP) and thyrotropin releasing hormone (TRH). In nearly all cases, surviving transplants were found. The grafts derived from mesencephalic raphé cells contained neurones which were immunoreactive to 5HT, or SP, but not both together. On average 4% of the total possible number of the available embryonic mesencephalic serotoninergic cells were found. A very dense outgrowth of 5HT positive fibres from the transplant was observed, extending up to 1.5 cm in both the caudal and rostral directions from the graft locus. Some SP immunoreactive fibres were also apparent near the implant. The grafts derived from the medullary transplant also contained 5HT-immunoreactive cells, comprising on average 25% of the total 5HT neurones available from the embryonic medullary primordium. In addition, neurones co-localizing 5HT together with SP and TRH were visible, closely reflecting the situation found in the medullary raphé in situ. Dense plexi of fibres containing 5HT-LI extended both caudally and rostrally up to 12–15 mm from the transplant. Outgrowth of SP and TRH varicose fibres was also demonstrable, although to a lesser degree than for 5HT. It was also possible to find many motoneurones surrounded by varicose fibres containing both 5HT and SP, in contrast to the situation with the mesencephalic grafts, where no such patterns of innervation were seen. The experiments indicate that the milieu of the spinal cord may compromise the survival of mesencephalic raphé 5HT neurones far more than of medullary serotonin cells. However, despite this effect on cell survival, the outgrowth of fibres from the remaining mesencephalic 5HT neurones was apparently unaffected by their ectopic position. Similarly, the transmitter content of both classes of raphé cells was largely unaltered, either by the transplantation process or by the environment into which they were placed. It is concluded that although the adult denervated spinal cord can selectively affect neuronal survival, it is incapable either of inducing in other serotoninergic cells placed within it the transmitter phenotype typical of medullary raphé neurones, or of causing those ectopically located 5HT cells to form connections appropriate to the descending serotonin fibres.

Key words: Transplantation – Medullary and mesencephalic raphé – Host spinal cord – Transmitter phenotypy – Fibre outgrowth – Selective neuronal death

Introduction

Grafting of neuronal tissue into the mammalian central nervous system has been documented since the beginning of the century (Saltykow 1905; Dunn 1917; Tidd 1932). It is only recently, however, that some reliability in the survival of the transplanted material has been achieved, and with it the possibility of evaluating in detail the morphological and functional integration of the graft with the nervous system.
of the host (see Björklund and Stenevi 1984, for review). Evidence has been accrued from a variety of experimental models indicating that the transplants may be sufficiently viable to reverse some lesion-induced, age-induced or genetic behavioural syndromes (Perlow et al. 1979; Björklund and Stenevi 1979; Gash et al. 1980; Dunnett et al. 1981a, b, 1982, 1985; Krieger et al. 1982; Gage et al. 1983, 1984b), or to attenuate specific neurochemical deficits (Freed et al. 1980; Schmidt et al. 1982, 1983; Björklund et al. 1983a). Although the existence of synaptic contacts has been demonstrated (Freund et al. 1985), the improvements in behaviour have not yet been shown to be dependent on synaptic transmission between host and transplant, and may merely reflect an overflow of neuroactive substances from the graft into the surrounding neuropil. Nevertheless, it is clear that a considerable two-way interaction occurs and that the host brain environment can exert trophic influences on the grafted neurones (Björklund and Stenevi 1981; Gage et al. 1983, 1984a). In other experimental systems, it has been shown that the environment may play an important role in determining the development and behaviour of neurones. For example, in vitro the constituents of the medium can change the transmitter phenotype of cultured sympathetic ganglion neurones from noradrenergic to cholinergic (Patterson and Chun 1977). In vivo, the locale (le Douarin et al. 1978; Kessler and Black 1982) and the activity of the afferent nerves (Kessler et al. 1981) both have profound trophic influences, particularly on the levels of the endogenous neurotransmitters.

Some serotoninergic neurones in the medulla oblongata of the rat are known to also contain substance P (SP) and/or thyrotropin releasing hormone (TRH) (Johansson et al. 1981). In contrast, serotoninergic cell bodies in the mesencephalic raphé nuclei have not been shown to store either SP or TRH, although cells with SP alone (Ljungdahl et al. 1978), but not TRH alone (Foster and Hökfelt, unpublished observations), have been observed. A second difference between these two cell groups is that those in the medullary raphé nuclei predominantly innervate the spinal cord, whereas those in the mesencephalon project mainly to diencephalic and telencephalic regions (Dahlström and Fuxe 1964, 1965; Fuxe and Jonsson 1974; Bowker et al. 1981, 1983; Tohyama et al. 1979; Björklund and Skagerberg 1982).

In view of the sensitivity of the transmitter complement of peripheral neurones to a variety of environmental influences, an attempt was made to discover if the same phenomenon also existed in the central nervous system. In addition, it was also desired to examine if survival or fibre outgrowth of the grafted serotoninergic cells was determined by their area of origin, or was influenced by the milieu in which they develop. Accordingly, suspensions of embryonic mesencephalic or medullary raphé neurones were grafted to three structures in the adult rat CNS with varying degrees of specificity, namely the striatum, hippocampus and spinal cord. In this