Colocalization of the cannabinoid receptor 1 and NO synthase in folliculo-stellate cells

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Introduction The hypophysial pars tuberalis (PT) is an important center that conveys photoperiodic information to neuroendocrine circuits involved in the control of seasonally changing body functions, like reproduction, metabolism and behaviour. In mammals, melatonin is used as an endocrine code for the length of the night and controls the production of messenger molecules representing the output from the PT (**Fig. 1**). These messengers appear to act both retrogradely (to the hypothalamus) and anterogradely (to the pars distalis of the pituitary; PD). A retrograde messenger from the PT is thyreotropin that, in the ependymal cells of the infundibular recess (tanycytes), regulates the transcription of type 2 and type 3 deiodinases to control the local concentration of trijodthyronine (T3) that subsequently affects gonadotropin releasing hormone (GnRH) secretion (Nakao et al. 2008; Ono et al 2008). Anterograde messengers from the PT are thought to control the secretion of adenohypophysial hormones, like prolactin, in a photoperiod-dependent manner, but have not yet been identified.

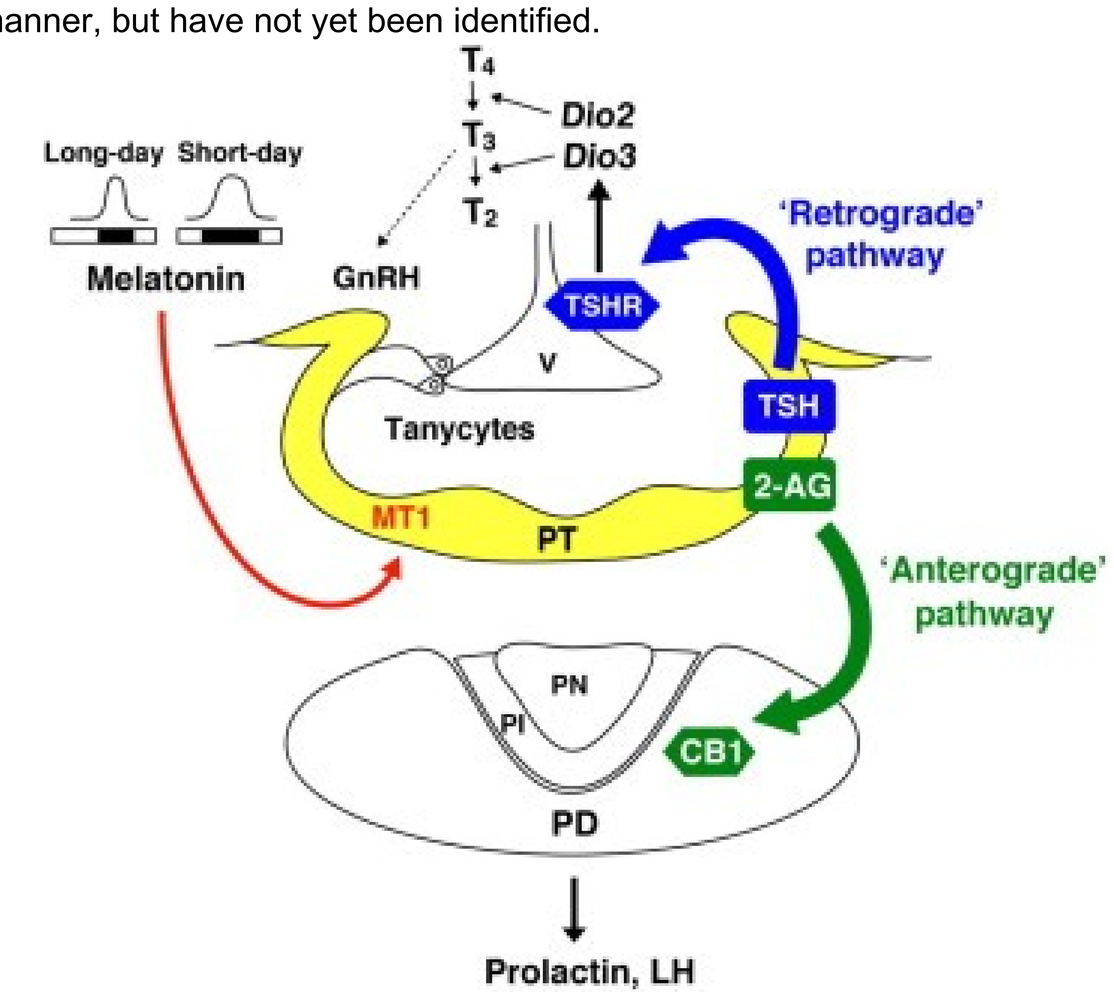

Fig. 1 Signaling pathways of messenger molecules produced in the rodent pars tuberalis (PT). 2-AG, 2-arachidonoylglycerol; CB1, cannabinoid receptor 1; Dio2 and Dio3, type 2 and 3 deiodinase; GnRH, gonadotropin releasing hormone (GnRH); LH, luteinizing hormone; MT1, type 1 melatonin receptor; PI, pars intermedia; PN, pars nervosa; T3, triiodothyronine; TSH, thyreotropin; TSHR, TSH receptor; V, third ventricle. From Yasuo and Korf, 2011.

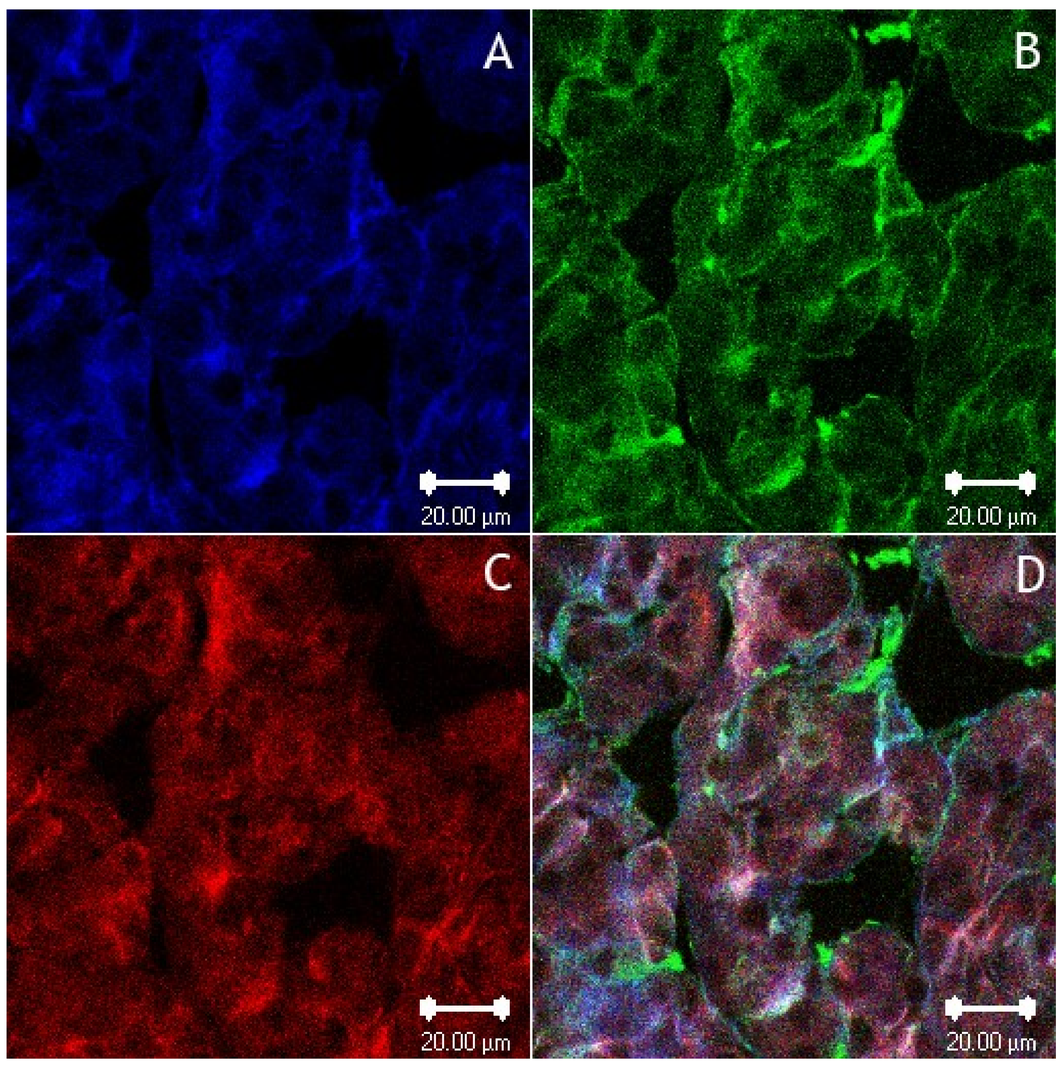

Fig. 2 Triple immunofluorescence staining of hamster pituitary cryosections. **A**, nitric oxide synthase (NOS); **B**, folliculo-stellate marker protein S-100; **C**, CB1 receptor; **D**, overlay of **A**, **B**, and **C**. 80% of the S-100 immunopositive cells are also immunoreactive for NOS. 45% of the S-100 immunopositive cells are also immunoreactive for CB1. 38% of the S-100 immunopositive cells are immunore-active for both NOS and CB1. The immunoreactions are located mostly in the cytoplasm.

Recently, endocannabinoids have been proposed to function as anterograde messengers since an endocannabinoid system has been found in the PT (Yasuo et al. 2010a, b). In principle, endocannabinoids may act either directly on the endocrine cells of the PD or indirectly via folliculo-stellate (FS) cells that are known to orchestrate adenohypophysial functions in response to various physiological stimuli. In the peresent study we performed immunocytochemical and immunochemical experiments to approach the question whether FS cells can mediate the effect of endocannabinoids in the PD via nitric oxide (NO) or the protein follistatin that is known to fullfill regulatory functions in the pituitary. Experiments were performed with pituitary cryostate sections obtained from hamster or with cells from the murine FS cell line TtT/GF.

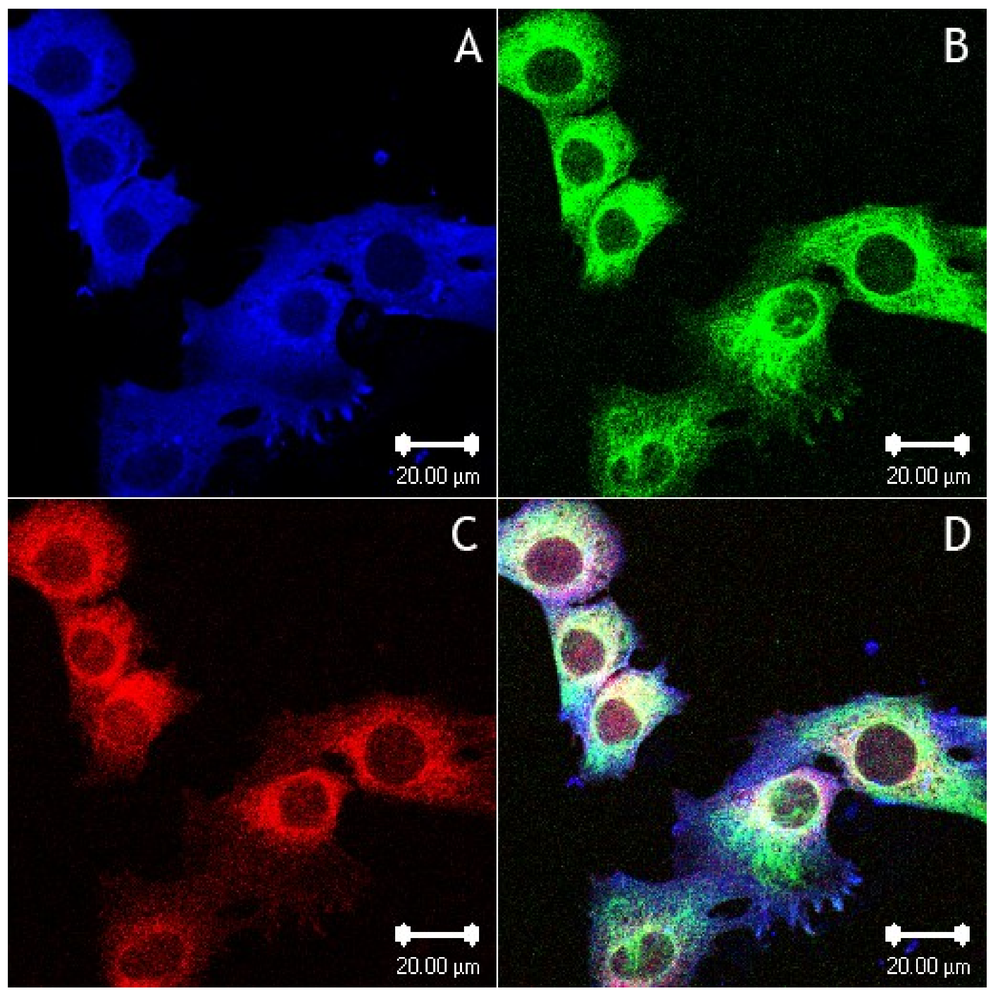

Fig. 3 Triple immunofluorescence staining of TtT/GF cells. **A**, nitric oxide synthase (NOS); **B**, S-100; **C**, CB1 receptor. **D**, overlay of **A**, **B**, and **C**. Colocalization of both NOS, S-100, and CB1 is observed in the cytoplasm of most of the TtT/GF cells.

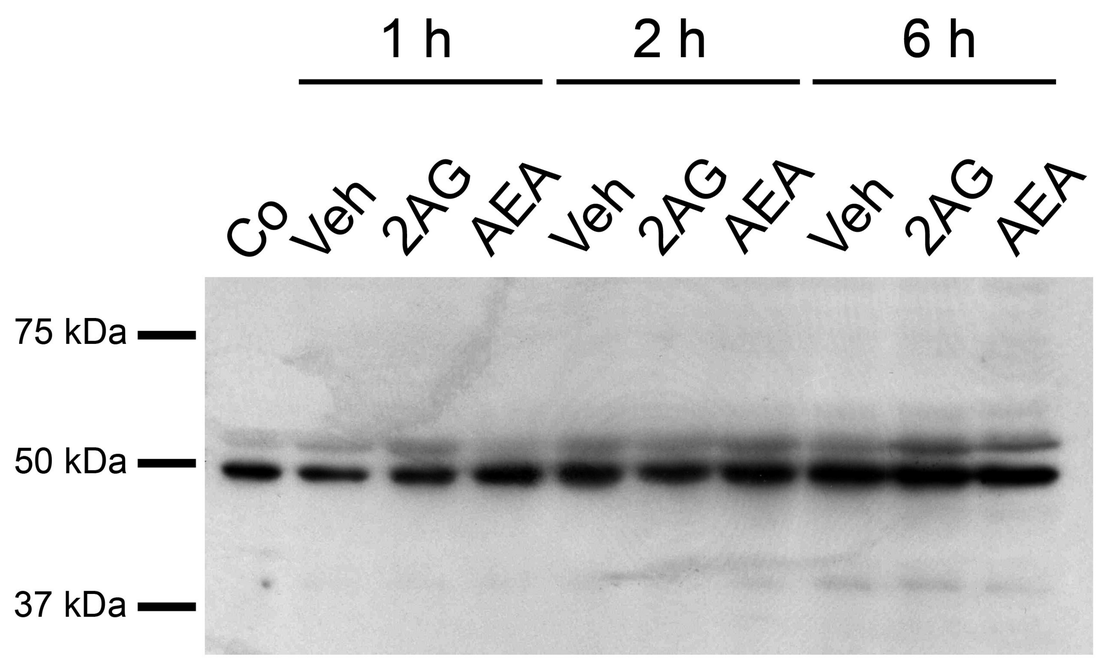

Fig. 4 Follistatin immunoblot. TtT-GF cells were stimulated with vehicle (Veh; DMSO), 10^{-7}M 2-arachidonoylglycerol (2-AG), or 10^{-7}M arachidonoyl-ethanolamine (AEA) for the indicated time intervals. The intensity of the immunosignal upon stimulation with endocannabinoids does not differ significantly from signals derived from vehicle-stimulated cells.

Conclusions The presence of CB1 receptors in a subpopulation of hamster folliculo-stellate cells and in the murine folliculo-stellate (FS) cell line TtT/GF supports the idea that FS cells mediate the effects of endocannabinoids to endocrine PD cells. Our dara show that apparently endocannabinoids do not act via follistatin. Interestingly, the colocalization of CB1 and NOS in FS cells indicates that NO may be involved in endocannabinoidergic signaling.

References

Nakao et al. (2008) Nature 452:317-322
Ono et al. (2008) PNAS 105:18238-18242
Yasuo et al. (2010a) Cell Tissue Res 342:273-281
Yasuo et al. (2010b) Cell Tissue Res 340:127-136
Yasuo and Korf (2011) Gen Comp Endocrinol172:15-22