A selective inhibitor of protein kinase A induces behavioural and neurological antidepressant-like effects in rats

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INTRODUCTION

The cyclic adenosine monophosphate (cAMP) signal transduction pathway has been shown to be closely involved in depression [2, 7]. While PKA remains the most widely described mediator of intracellular cAMP signals, the pivotal role of PKA as a mediator of cAMP signalling has been challenged by the discovery of cyclic nucleotide-gated ion channels and a novel guanine nucleotide exchange factor regulated directly by cAMP (Epac) [1]. Epac is a guanine exchange factor for Rap-1 and has been shown to participate in certain depression-related physiological processes such as neurotransmitter release [6] and neuroplasticity [4]. In turn, Rap-1 activates the mitogen-activated protein kinase (MAPK) cascade that regulates the phosphorylation of a variety of transcription factors including CREB [5, 9]. Therefore, Epac may function as an alternate pathway through which cAMP can induce antidepressant-like activity. In line with this notion, both Epac and Rap-1 have been associated with clinical depression [3, 8].

OBJECTIVES

In this study we investigated:

- the relative importance of cAMP signalling via PKA versus Epac in mood-regulation by evaluating the effect of Rp-8-Br-cAMPS (a PKA inhibitor with a relatively low affinity for Epac) in the rat forced swim test (FST);
- the action of this drug on the phosphorylation status of CREB, which is thought to play an important role in depression and antidepressant treatment;
- cAMP and cGMP levels in several brain regions (since Rp-8-Br-cAMPS also acts as a inhibitor of phosphodiesterase 1 (PDE1); and
- whether any observed effects may involve the activation of cGMP-dependent protein kinase (PKG), by using an inhibitor of this enzyme (Rp-8-Br-PET-cGMPS).

MATERIALS AND METHODS

Surgery: Male Sprague Dawley rats were implanted with an i.c.v. guide cannula to the right lateral ventricle and were allowed 7 days for recovery.

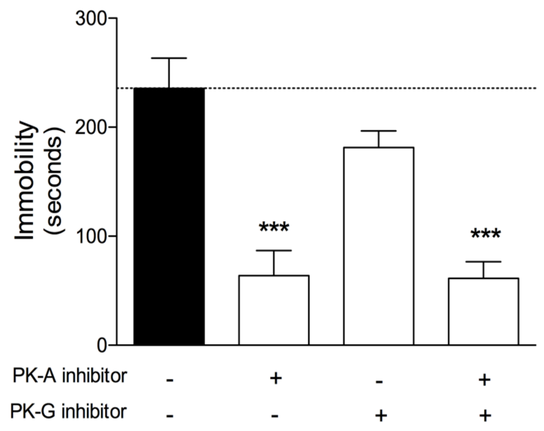
Forced swim test and treatment: Animals were subjected to a 15 minute pre-swim followed by a 5 min test swim 24 hours later. Three 5 µl infusions of vehicle (Ringer's solution) or drug solution (Rp-8-Br-cAMPS (100 nmol), Rp-8-Br-PET-cGMPS (1 nmol) or both) were administered at 24, 6 and 1 hour before the final swim. Rats were decapitated and their brains dissected immediately following the test.

Neurobiological measurements: Western blotting was used for the measurement of total and phosphorylated CREB levels in the cerebellum, whereas low pH ELISA kits (Sigma) were used for the measurement of cAMP and cGMP levels in hippocampus, frontal cortex and cerebellum regions. *Statistics*: All data were analysed using one-way analyses of variance (ANOVA) followed by Dunnett's multiple comparison tests. All animal procedures were approved by the Danish National Committee for Ethics in Animal Experimentation (2007/561-1378).

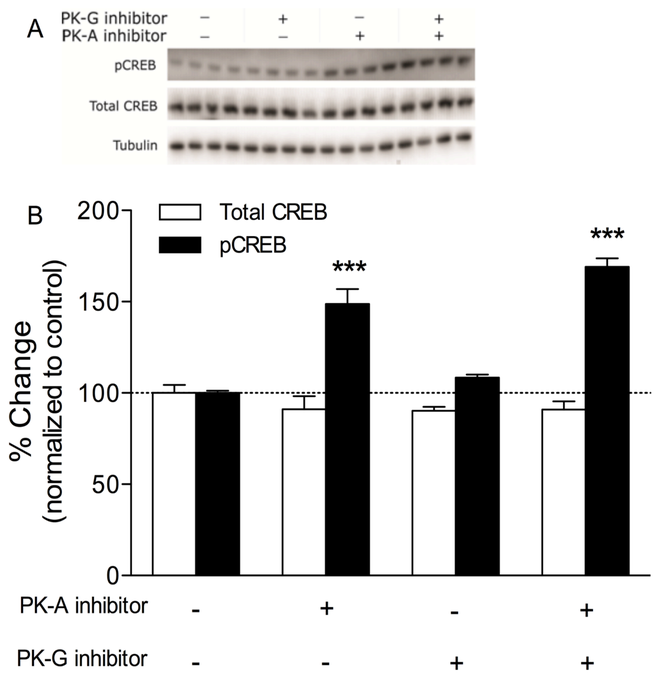
CONFLICT OF INTEREST

There are no conflicts of interest to declare.

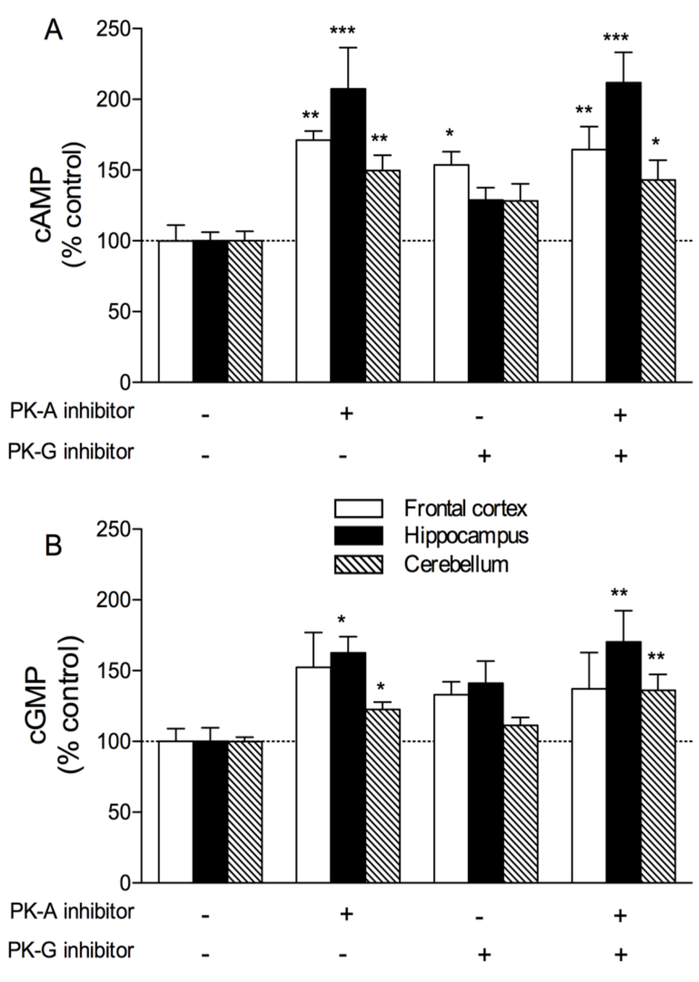
RESULTS



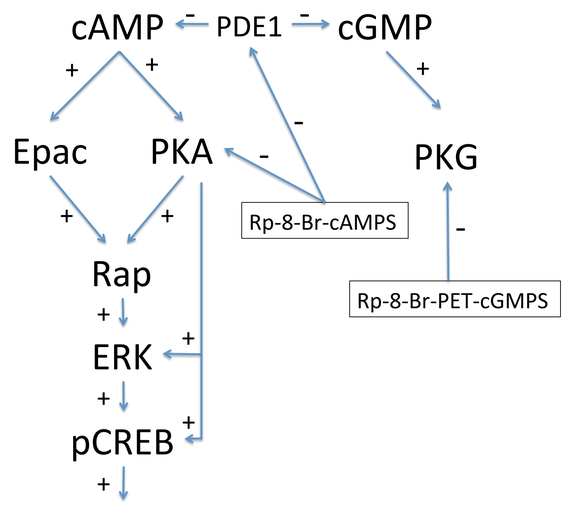
Immobility in the FST is reduced following sub-acute i.c.v. infusion of an inhibitor of PKA (Rp-8-Br-cAMPS). This effect is unaffected by an inhibitor of PKG (Rp-8-Br-PET-cGMPS). Results are presented as the mean ± SEM. *** $P < 0.001$ ($n = 7$ for control group and $n = 4$ for treatment groups).



CREB phosphorylation is increased in cerebellar nuclear fractions following sub-acute i.c.v. infusion of an inhibitor of PKA (Rp-8-Br-cAMPS). Total CREB protein was unchanged and an inhibitor of PKG (Rp-8-Br-PET-cGMPS) did not affect the action of Rp-8-Br-cAMPS. Representative immunoreactive bands from immunoblots were quantified by densitometry, normalized to tubulin, and expressed as the mean ± SEM of control. *** $P < 0.001$ ($n = 4$ for all groups).



cAMP and cGMP levels are increased in the frontal cortex, hippocampus and cerebellum following sub-acute i.c.v. infusion of Rp-8-Br-cAMPS. An elevation in cAMP in the frontal cortex was the only significant effect produced by Rp-8-Br-PET-cGMPS on cyclic nucleotide levels and this drug did not modify the action of Rp-8-Br-cAMPS. Results are expressed as percentage of vehicle-treated control and presented as the mean ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ ($n = 6$ - 8 for the control group and $n = 3$ - 4 for treatment groups).



KEY FINDINGS

- Sub-acute i.c.v. infusion of Rp-8-Br-cAMPS, an inhibitor of PKA but not Epac, induces antidepressant-like effects in rats, namely:
  - reduced immobility in the FST, and
  - increased central CREB phosphorylation.
- Rp-8-Br-cAMPS induces an elevation in central cAMP and cGMP levels (likely caused by its PDE1 inhibiting action).
- The antidepressant-like effects induced by Rp-8-Br-cAMPS was unaffected by Rp-8-Br-PET-cGMPS, an inhibitor of protein kinase G.

CONCLUSIONS

- Protein kinase A may not be the only mediator of cAMP-induced antidepressant activity, but other mechanisms (such as the activation of Epac) may be an additional important mediator for cAMP-mediated antidepressant activity.
- It is likely that there exists a dual regulation by PKA and Epac in depression and antidepressant action.
- This study identifies Epac as a promising novel antidepressant target.

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