Muscarinic Acetylcholine Receptors Inhibit Src Family Tyrosine Kinase Phosphorylation in the Rat Striatum

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INTRODUCTION

- Acetylcholine (ACh) is an important neurotransmitter in the mammalian brain
- ACh binds the muscarinic acetylcholine receptor (mAChR), which regulates intracellular protein kinases
- Fyn and Src are two members of the Src family kinase (SFK) , which are highly expressed in broad brain regions, including the striatum[1]
- Striatum is an area in the forebrain critical for cognitive functioning, reward, mood, and movement[1]
- Since the striatum is also among brain regions with a high level of mAChR expression[2], it is intriguing to investigate whether mAChRs are involved in the regulation of Fyn and Src
- In this study, we initiated a first effort to experimentally determine whether pharmacological blockade of mAChRs in the adult rat striatum *in vivo* caused a change in phosphorylation of Fyn and Src at a specific tyrosine site, tyrosine 416 (Y416)
  - Phosphorylation at Y416 causes Fyn and Src kinase activation[1]

METHODS

- Adult male rats were randomly divided into four groups with four rats per group. Animal use and care were approved by IACUC.
- Scopolamine was injected at 5 mg/kg via an intra-peritoneal (i.p.) injection into each rat in each group. Rats were sacrificed at different time points (0, 7.5, 15, or 30 min) after drug injection.
- Two subdivisions of the striatum, the caudate putamen (CPu) and nucleus accumbens (Nac), were dissected. Western blot was conducted using antibodies against phosphorylated SFK at Y416 (pY416), Fyn, or Src.
- One-way ANOVA statistical analysis was performed. $P < 0.05$ was considered significant difference.

RESULTS

Fig. 1. Effects of scopolamine (5 mg/kg, i.p.) on phosphorylation of Fyn/Src proteins in the rat caudate putamen.

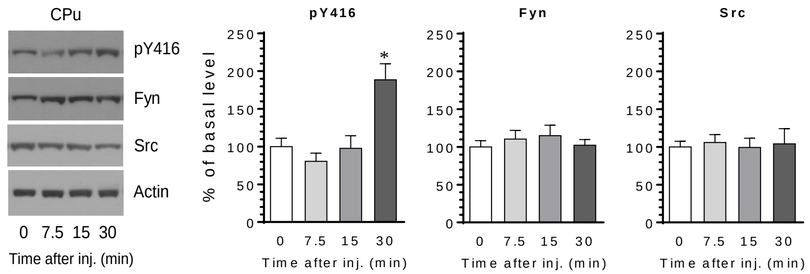
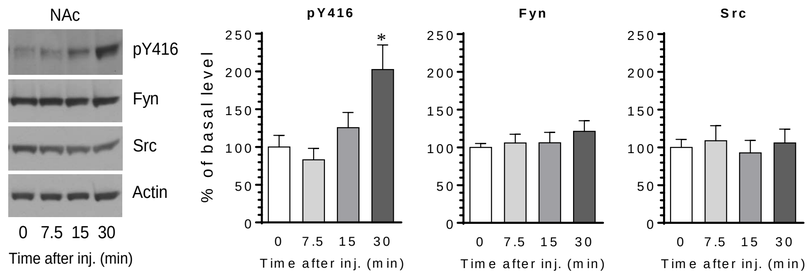


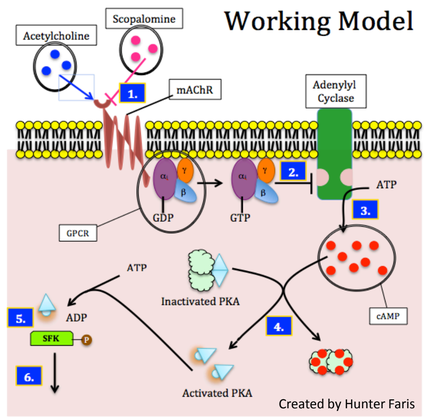
Fig. 2. Effects of scopolamine (5 mg/kg, i.p.) on phosphorylation of Fyn/Src proteins in the rat nucleus accumbens (NAc).



* = significance

SUMMARY

- Scopolamine induced an increase in SFK Y416 phosphorylation in the Cpu at the 30 min interval
- Scopolamine also induced an increase in SFK Y416 phosphorylation in the Nac at the 30 min interval
- Scopolamine did not alter levels of total Fyn and Src proteins in both regions



1. Scopolamine blocks mAChR
2. mAChR can't activate $G\alpha_i$ which would inhibit adenylyl cyclase
3. Adenylyl cyclase stays active & makes cAMP
4. cAMP activates PKA
5. PKA phosphorylates SFK
6. Activated SFK causes effects downstream (molecular mechanisms poorly understood)

mAChR = muscarinic acetylcholine receptor; GPCR = G-protein coupled receptor (α_i); GDP = guanosine diphosphate; GTP = guanosine triphosphate; cAMP = cyclic adenosine monophosphate; ATP = adenosine triphosphate; ADP adenosine diphosphate; PKA = protein kinase A; SFK = Src Family Kinase; P = phosphate

CONCLUSION/SIGNIFICANCE

- Since the mAChR antagonist increased SFK phosphorylation, mAChRs exert an inhibitory effect on SFK activity in striatal neurons.
- The mAChR antagonist had no effect on total cellular levels of Fyn and Src. Thus, mAChRs primarily regulate the phosphorylation reaction but not expression of Fyn and Src proteins.
- The mAChR-regulated Fyn and Src activity may play a role in controlling normal neuronal and synaptic activities in the striatum.

CREDITS/DISCLOSURE/REFERENCES

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2. Levey AI, Kitt CA, Simonds WF, Price DL, Brann MR. 1991. Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies. J Neurosci 11:3218-26.

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