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SST_5 RECEPTORS ACT VIA PROTEIN KINASE C AND SRC TO ACTIVATE NMDA RECEPTORS IN PRESENCE OF PHYSIOLOGICAL AMOUNTS OF MG^{2+} IONS

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A recent paper proposed that somatostatin (SRIF), inactive on its own, permits activation of NMDA receptors presynaptically located on rat hippocampal noradrenergic nerve terminals, allowing the occurence of a NMDA-dependent exocytotic-like [^{3}H]NA release in presence of physiological amounts of Mg^{2+}ions (i.e. 1.2 mM). The SRIF-mediated NMDA receptor-activation involves receptor-receptor interaction (R-R interaction) between somatostatinergic receptors belonging to the sst_5 subtype and NMDA receptors, co-localized on the same noradrenergic nerve terminals and it is mediated by PKC-dependent phosphorylation of NMDA subunits. Since the R-R-mediated NMDA-activation might depend on either direct or indirect (cytosolic tyrosin kinase (Src)-involving) PKC-dependent phosphorylation.of the NMDA subunits, we have here tried to evaluate the involvement of Src_s in the above described R-R interaction.

The experimental approach used is the superfusion of isolated (pinched off) nerve terminals (synaptosomes). Rat hippocampal synaptosomes were labelled with [^{3}H]NA (30nM) in a rotary water bath at 37°C for 15 min. Identical aliquots of the synaptosomal suspension were then layered at the bottom of 20 thermostatated chambers and superfusion was carried out at the flow rate of 0.5ml/min. After 36 min of superfusion to equilibrate the system, eight consecutive 1-min fractions were collected and counted for radioactivity. Agonists were added at t=38 min , while antagonists were added 8 min before agonists.

The amount of [^{3}H]NA released into each 1-min fraction collected was expressed as a percentage of total synaptosomal tritium at the start of the fraction collected. The effects of the agonists or antagonists under study were evaluated by performing the ratio between the percentage efflux in the fractions after the first and the efflux in the first fraction collected in the same treatment. This ratio was compared to the corresponding ratio obtained under control condition. One-way ANOVA test was used to analyse statistical differences followed by Newmann-keuls test as appropiate. Exposure of hippocampal synaptosomes to 1nM SRIF + 100µM NMDA + 1µM glycine increases the basal [^{3}H]NA release (+68.7 ± 8.8, n=5) and the potentiating effect is totally antagonized by the presence of 10µM CGS 19755, a competitive antagonist of NMDA receptor (+ 10.4 ± 2.3, n=6; $P<0.01$). The [^{3}H]NA release induced by 1nM SRIF + 100µM NMDA + 1µM glycine is prevented by the presence of 20µM genestin, a broad spectrum antagonist of Src family.

Similar results were obtained when evaluating the effect of lavendustin A, a non selective Src inhibitor and PP2, a selective inhibitor of the Src-subtype fyn (1nM SRIF + 100µM NMDA + 1µM glycine= + 78.9 ± 8.6; 1nM SRIF + 100µM NMDA + 1µM glycine+ 50µM lavendustin A= + 47.9 ± 8.3, $P<0.05$; 1nM SRIF + 100µM NMDA + 1µM glycine + 10µM PP2= + 31.1 ±

5.1, P<0.05). At the concentration used the antagonists did not affect on their own the spontaneous release of [^{3}H]NA from hippocampal synaptosomes.
It is concluded that SRIF-mediated activation of NMDA receptors modulating the [^{3}H]NA release from hippocampal synaptosomes depends on an indirect PKC-dependent Src-mediated phosphorylation of the NMDA receptor, probably involving phosphorylation of tyrosinic residues located in the NR2B subunit.
