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# Subtypes of Brain Somatostatin Receptors Couple to Multiple Cellular Effector Systems

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**SUMMARY**

To investigate whether somatostatin (SRIF) receptor subpopulations mediate different physiological actions of SRIF, we tested the effects of SRIF and the SRIF agonists MK 678 and CGP 23996 on different biological responses in rat neocortical neurons in culture. Neocortical cells in culture express SRIF receptors that can be labeled with $^{125}$I-MK 678 and $^{125}$I-CGP 23996. Pharmacological analysis of the binding sites indicates that the radioligands label SRIF receptor subtypes with distinct pharmacological characteristics. These receptor subpopulations are similar to those expressed in adult rat brain. SRIF, MK 678, and CGP 23996 are able to inhibit forskolin-stimulated adenylate cyclase activity in rat neocortical membranes by 25–30%. Furthermore, they inhibit a high voltage-activated $Ca^{2+}$ current in rat neocortical neurons in culture by 25–35%. Both SRIF and MK 678 potentiate a delayed rectifier $K^+$ current in rat neocortical neurons in culture by 25–30%. In contrast, high concentrations of CGP 23996 do not alter the $K^+$ current. In cells that do not respond to CGP 23996, MK 678 increases the delayed rectifier $K^+$ current. The findings of these studies indicate that rat neocortical neurons in culture express functionally distinct SRIF receptor subtypes that can be differentially activated by SRIF agonists.

The neuropeptide SRIF exerts diverse physiological actions in the nervous system (1). In the basal ganglia, SRIF has been postulated to play a role in the regulation of movement (2–4). It has also been suggested to influence cognitive processes (5–7) through its actions in limbic structures. At the cellular level, SRIF has complex effects on neuronal firing activity. It causes neuronal depolarization (8–10) and hyperpolarization (11) and potentiates $K^+$ currents (12–14). SRIF also inhibits $Ca^{2+}$ conductance in cerebrocortical (15) and sympathetic neurons (16). In addition to its modulation of ionic conductances, SRIF inhibits adenylate cyclase activity in brain (1, 17).

SRIF induces its cellular actions by interacting with membrane-bound receptors. The findings of previous studies have suggested that multiple subtypes of SRIF receptors are expressed in brain. The results of receptor binding studies suggest that SRIF receptor subtypes express similar affinities for the native peptides SRIF and SRIF-28 but differing affinities for synthetic SRIF analogs such as SMS-201-995 and MK 363-823 (18, 19). Physical analysis of SRIF receptors using photo-cross-linking techniques also indicates that multiple SRIF receptors are expressed in brain (20). Recently, we reported that rat brain expresses at least two subpopulations of SRIF receptors (21, 22). One receptor population has high affinity for SRIF and the SRIF agonist MK 678, whereas the other has high affinity for SRIF and CGP 23996 but has no affinity for MK 678. The pharmacological characteristics of these two SRIF receptors are different, as are their regional distributions, suggesting that they may mediate distinct biological actions of SRIF.

Subtypes of receptors for a number of neurotransmitters have been found to mediate distinct biological actions. For example, M1 muscarinic receptors mediate the stimulation of phosphatidylinositol turnover by acetylcholine, whereas M2 muscarinic receptors mediate cholinergic inhibition of adenylate cyclase activity (23). Furthermore, D1 and D2 dopamine receptors mediate opposing actions of dopamine on adenylate cyclase activity (24). The opposing actions of adrenaline on cAMP synthesis are mediated by $\alpha_2$- and $\beta$-adrenergic receptors (25). However, despite the multitude of physiological actions induced by SRIF, relatively little is known about whether different biological effects of SRIF are mediated by distinct receptor subpopulations.

To test whether subtypes of SRIF receptors mediate different physiological responses of SRIF, we have investigated the actions of SRIF, MK 678, and CGP 23996 on multiple cellular responses in rat neocortical neurons in culture. These cells have served as a model system to study SRIF physiology. They express SRIF-like immunoreactivity and release SRIF in a

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**ABBREVIATIONS:** SRIF, somatostatin; SRIF-28, somatostatin-28; $I_K$, delayed rectifier $K^+$ current; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis($\beta$-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid.

$Ca^{2+}$-dependent manner (10, 26, 27). Previous studies have shown that SRIF specifically potentiates $I_K$ (14, 28), inhibits a voltage-dependent $Ca^{2+}$ current (15), and diminishes adenylate cyclase activity in these cells (14). The results of our present study indicate that some cellular responses to SRIF in rat neocortical neurons in culture are selectively mediated by different SRIF receptor subtypes.

## Experimental Procedures

**Materials**. SRIF, SRIF-28, and SRIF-28[1–14] were obtained from Bachem (CA). SMS-201-995 (D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-ol) was a gift of Dr. J. C. Reubi of Sandoz (Basel, Switzerland). MK 678 (cyclo-*N*-Me-Ala-Tyr-D-Trp-Lys-Val-Phe) and MK 363-301 (Pro-Phe-D-Trp-Lys-Thr-Phe) were gifts from Drs. R. Hirschman and D. Veber, Merck (Rahway, NJ). CGP 23996 [des-Ala$^1$-Gly$^2$-(desamino-Cys$^3$, Tyr$^{11}$)-3,14-dicarba-SRIF] was a gift from Dr. B. Petrack, Ciba Geigy (Rahway, NJ). CGP 23996 and MK 678 were iodinated as previously described (21).

**Cell culture**. Primary rat cerebrocortical cultures were prepared as previously described (14, 15, 28). Briefly, cerebral cortices were removed from 17-day-old fetuses, dissociated in 0.03% trypsin-Dulbecco's modified Eagle's medium for 40 min at 37°, and then reincubated for 20 min in Hank's Balanced Salt solution. Cells were subsequently dispersed by trituration and plated onto 35-mm Petri dishes containing polylysine-coated coverslips or onto 60-mm polylysine-coated Petri dishes, at a density of 675 or 530 cells/mm$^2$, respectively, in growth medium (Dulbecco's modified Eagle's medium supplemented with 10% Hyclone calf serum, 10% Ham's F 12 with glutamine, and 50 units/ml penicillin-streptomycin). Cultures were maintained at 36–37° in a humidified 5% $CO_2$ incubator. Proliferation of glial cells was prevented by the addition of cytosine arabinoside to the cultures 7–10 days after the initial subculturing. Rat neocortical neurons cultured for 2–5 weeks were used in the present study.

**SRIF receptor binding**. Membranes from cultured cortical cells were harvested in 50 mM Tris·HCl buffer, pH 7.4, containing 200 μg/ml bacitracin, 10 μg/ml leupeptin, 10 μg/ml pepstatin, 0.5 μg/ml aprotinin, 5 mM $MgCl_2$, and 1 mM EGTA (buffer 1), and were centrifuged at 20,000 × *g* for 15 min at 4°. The pellet was homogenized in buffer 1, using a Brinkman Polytron (setting 2.5 for 30 sec), and was used in the receptor binding assay or the adenylate cyclase assay.

For the binding assays, membranes were incubated with either $^{125}$I-CGP 23996 (specific activity, 505 Ci/mmol) or $^{125}$I-MK 678 (specific activity, 2200 Ci/mmol) in buffer 1, in the presence or absence of unlabeled peptides, in a total volume of 200 μl for 90 min at 25°. The binding reaction was terminated by vacuum filtration over GF/C glass fiber filters (Whatman), which had been presoaked in 0.5% polyethylenenimine and 0.1% bovine serum albumin, using a Brandel harvester. The filters were washed with 15 ml of cold Tris·HCl, pH 7.8, and the bound radioactivity was analyzed in a γ-counter (80% efficiency). Specific binding was defined as the total $^{125}$I-CGP 23996 or $^{125}$I-MK 678 tissue binding minus the amount bound in the presence of 100 nM SRIF. Data from these studies were used to generate the inhibition curves. $IC_{50}$ values were obtained from curve-fitting involving nonlinear least-squares regression analysis. The analysis was performed by the mathematical modeling program NEWFITSITES, available on the National Institutes of Health-sponsored PROPHET system. The dissociation constant ($K_D$) for radioligand binding and the density of each receptor subtype ($B_{max}$) was determined by linear analysis of the saturation isotherms, using mathematical modeling programs also available in the PROPHET program. Figs. 1–4, describing the radioligand binding data, contain inhibition curves, saturation isotherms, or linearizations of the saturation isotherms, which were all generated by the curve-fitting programs of the PROPHET system.

**Adenylate cyclase activity**. Adenylate cyclase activity was measured using the procedure described by Mahy *et al.* (29). The adenylate cyclase activity was assayed by addition of 50 μl of rat neocortical cell membranes (20–30 μg) to 200 μl of an incubation medium. The final incubation mixture contained 100 mM NaCl, 30 μM GTP, 2 mM $MgCl_2$, 50 μM ATP, 0.1 mM cAMP, 0.5 mM isobutylmethylxanthine, 100 μg of creatine phosphokinase, 5 mM creatine phosphate, $10^6$ to 2 × $10^6$ cpm of [α-$^{32}$P]ATP (NEN), with or without 10 μM forskolin, and SRIF or various SRIF analogs. The incubation was carried out for 6 min at 30° and stopped by the addition of 150 μl of 10% sodium dodecyl sulfate, 5 mM ATP, in 50 mM Tris·HCl, pH 7.4. [$^3$H]cAMP (10,000 cpm) was added to the tubes to determine the recovery rate through the extraction procedure. The conversion of [$^{32}$P]ATP to [$^{32}$P]cAMP was measured as described by Salomon *et al.* (30).

**Electrophysiological recordings**. Whole-cell patch-clamp recordings from rat central nervous system neurons in culture were obtained as described previously (14, 15, 28). For analysis of voltage-dependent $Ca^{2+}$ currents, the extracellular bath was a HEPES-buffered saline containing 130 mM NaCl, 20 mM tetraethylammonium chloride, 5 mM 4-aminopyridine, 3 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, 5 μM tetrodotoxin, and 10 mM glucose (pH 7.3). The patch electrodes contained 130 mM CsCl, 2 mM $MgCl_2$, 5 mM MgATP, 10 mM EGTA, 20 mM tetraethylammonium chloride, 5 mM glucose, and 5 mM HEPES (pH 7.2). For the studies to examine $I_K$, the external solutions used during the recordings contained 145 mM NaCl, 3 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, and 8 mM glucose (pH 7.3). The $K^+$ current was isolated using 5 μM tetrodotoxin and 5 mM $CoCl_2$ to block $Na^+$ and $Ca^{2+}$ currents, respectively. Patch electrodes contained a solution of 140 mM KCl, 1 mM $MgCl_2$, 10 mM EGTA, and 10 mM HEPES (pH 7.3). Impaled cells were externally miniperfused with medium containing known concentrations of peptides, by pressure perfusion from micropipettes with 5–15-μm tip diameters placed within 10–50 μm from the cell. This system allows almost total replacement of the medium around the cell.

Student's unpaired *t* test was used to determine whether differences were statistically significant in the electrophysiology and adenylate cyclase assays. Values are presented as the mean ± standard error of multiple determinations.

## Results

**Receptor binding**. Previous studies have shown that the potent SRIF agonist MK 678 binds with high affinity and selectivity to a subpopulation of rat brain SRIF receptors (21, 22). To determine whether the neocortical cells in culture express this SRIF receptor subtype, the characteristics of $^{125}$I-MK 678 binding to neocortical membranes were determined. The equilibrium binding of $^{125}$I-MK 678 to rat neocortical cell membranes was saturable (Fig. 1). Analysis of the saturation isotherms revealed that $^{125}$I-MK 678 bound to a single site, with a $K_D$ of 0.25 ± 0.1 nM and a $B_{max}$ of 60 ± 8 fmol/mg of protein. $^{125}$I-MK 678 specifically bound to SRIF receptors in rat neocortical membranes. $^{125}$I-MK 678 binding was potently and effectively inhibited by SRIF, SRIF-28, and MK 678, as well as by the newly developed SRIF agonists SMS-201-995 (19, 31) and MK 363-301 (21) (Fig. 2). The $IC_{50}$ values for the ability of these peptides to inhibit high affinity $^{125}$I-MK 678 binding were 0.36 ± 0.05 nM, 0.48 ± 0.06 nM, 0.12 ± 0.01 nM, 0.67 ± 0.22 nM, and 6.6 ± 1.1 nM, respectively. Furthermore, CGP 23996 also completely displaced $^{125}$I-MK 678 binding to SRIF receptors, with an $IC_{50}$ value of 2.28 ± 0.1 nM. The inactive SRIF analog SRIF-28[1–14] did not displace specific $^{125}$I-MK 678 binding to the SRIF receptors (Fig. 2).

In previous studies, we showed that brain SRIF receptors can be labeled by the potent agonist $^{125}$I-CGP 23996 (21, 22). Using a limited radioligand concentration range (0.1–1.5 nM), $^{125}$I-CGP 23996 bound to cortical membranes in a saturable manner (Fig. 3). Analysis of high affinity $^{125}$I-CGP 23996 bind-

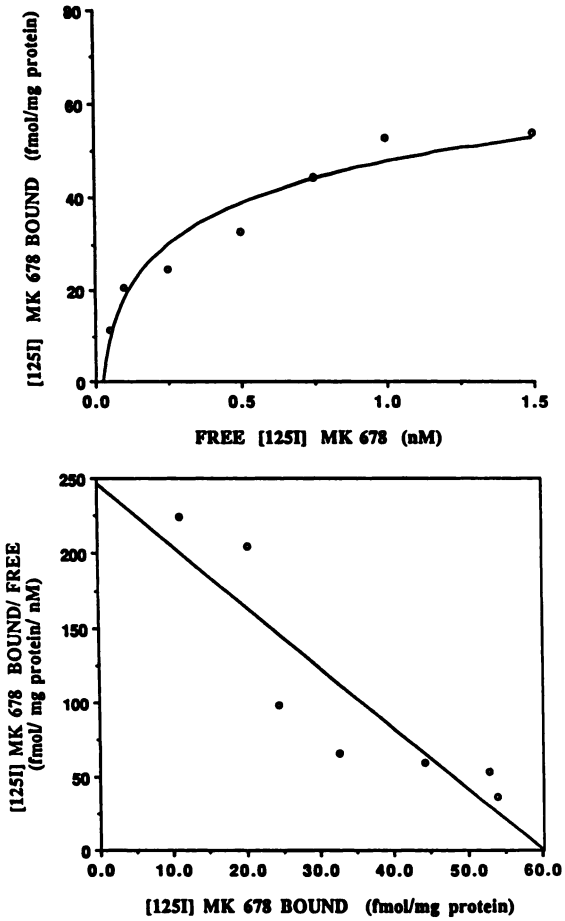


**Fig. 1.** Saturable binding of $^{125}$I-MK 678 to SRIF receptors in rat neocortical cell membranes. Rat neocortical membranes were incubated for 90 min at 25° with varying concentrations of $^{125}$I-MK 678, in the presence or absence of 100 nM SRIF, in order to determine specific $^{125}$I-MK 678 binding (○). *Upper*, saturation isotherm of a representative experiment; *lower*, linearization of the saturation isotherm data. Analysis of the saturable binding of $^{125}$I-MK 678 to neocortical SRIF receptors in three different experiments in different tissue cultures revealed that $^{125}$I-MK 678 bound to a single site, with an average $K_D$ value of 0.25 ± 0.1 nM and a $B_{max}$ of 60 ± 8 fmol/mg of protein.

ing to SRIF receptors indicates that the ligand binding was best fit to a single-site interaction, with a $K_D$ of 0.44 ± 0.2 nM and a $B_{max}$ of 27 ± 6 fmol/mg of protein (Fig. 3). Furthermore, CGP 23996 inhibited $^{125}$I-CGP 23996 binding with high affinity, with an $IC_{50}$ value of 1.4 ± 0.5 nM (Fig. 4). However, CGP 23996 appears to be able to interact with multiple SRIF receptors in neocortical membranes, because the synthetic SRIF agonist SMS-201-995 inhibited $^{125}$I-CGP 23996 binding in a biphasic manner, with $IC_{50}$ values 6.9 ± 3.6 nM and 1034 ± 322 nM (Fig. 4). In contrast, SRIF and SRIF-28 inhibited $^{125}$I-CGP 23996 binding in a monophasic manner, with $IC_{50}$ values of 0.31 ± 0.01 nM and 0.21 ± 0.03 nM, respectively. These latter findings suggest that SRIF receptor subtypes have equal affinities for the native peptides but different affinities for the synthetic compound SMS-201-995. Similar findings have been reported by Tran *et al.* (18) regarding the ability of SMS-201-995 to inhibit $^{125}$I-Tyr$^{11}$-SRIF or $^{125}$I-Leu$^{8}$-D-Trp$^{22}$-Tyr$^{25}$-SRIF-28 binding to rat brain. In those studies, it was found that SMS-201-995 was able to inhibit radioligand binding to rat brain membranes in a biphasic manner, whereas SRIF and SRIF-28 inhibited radioligand binding in a monophasic manner. The absence of biphasic $^{125}$I-CGP 23996 saturation curves is most likely due to the preferential labeling of a high affinity site with the concentrations of $^{125}$I-CGP 23996 used in our studies. Higher radioligand concentrations cannot be used, due to the large nonspecific component of total $^{125}$I-CGP 23996 binding at the higher radioligand concentrations. Therefore, accurate estimations of the $B_{max}$ of $^{125}$I-CGP 23996 binding to its second site in neocortical membranes cannot be made. Similarly, whereas previous studies (18) have reported that subtypes of brain SRIF receptors exist with differing affinities for SMS-201-995, saturation analysis of the binding of radioactive SMS-201-995 analogs to rat brain receptors revealed that the peptide interacted with only a single high affinity site (19), similar to what we have observed with $^{125}$I-CGP 23996.

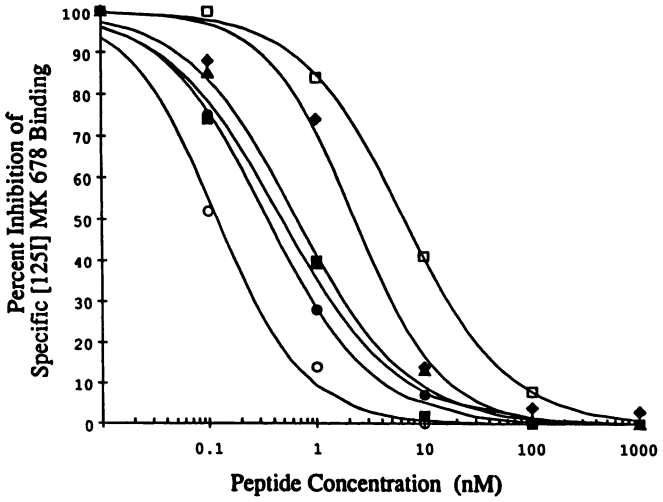


**Fig. 2.** Specificity of $^{125}$I-MK 678 binding to SRIF receptors in membranes from rat neocortical cells in culture. $^{125}$I-MK 678 (0.05 nM) binding to rat neocortical membranes was inhibited by varying concentrations of SRIF (●), SRIF-28 (■), SMS-201-995 (▲), MK 678 (○), MK 363-301 (□), and CGP 23996 (♦). These are the averaged results of three different experiments done on different tissue cultures.

The SRIF receptor subtypes labeled by $^{125}$I-CGP 23996, in addition to having differing affinities for SMS-201-955, also exhibited differing affinities for MK 678. MK 678 and its structural analog MK 363-301 only partially displaced $^{125}$I-CGP 23996 binding. Maximally, only 30% of SRIF-displacable $^{125}$I-CGP 23996 binding could be inhibited by the cyclohexapeptides. The $IC_{50}$ values for MK 678 and MK 363-301 inhibition of $^{125}$I-CGP 23996 binding were 0.06 ± 0.01 nM and 0.49 ± 0.01 nM, respectively. This partial displacement of binding may represent $^{125}$I-CGP 23996 binding to the SRIF receptor that expresses high affinity for MK 678 and CGP 23996, which is selectively detected by the $^{125}$I-MK 678 binding assay. The $^{125}$I-CGP 23996 binding sites not displaced by MK 678 represent a different SRIF receptor, inasmuch as radioligand binding to this site was effectively and potently inhibited by SRIF and SRIF-28 (Fig. 4).

**Functional responses mediated by SRIF receptors.** The binding studies revealed pharmacologically distinct SRIF receptors in rat neocortical membranes. To investigate whether SRIF receptor subtypes may mediate distinct functions of SRIF, we tested SRIF, MK 678, and CGP 23996 for their effects on adenylate cyclase activity and $Ca^{2+}$ and $K^+$ currents in the neocortical cells.

SRIF inhibited 10 μM forskolin-stimulated adenylate cyclase

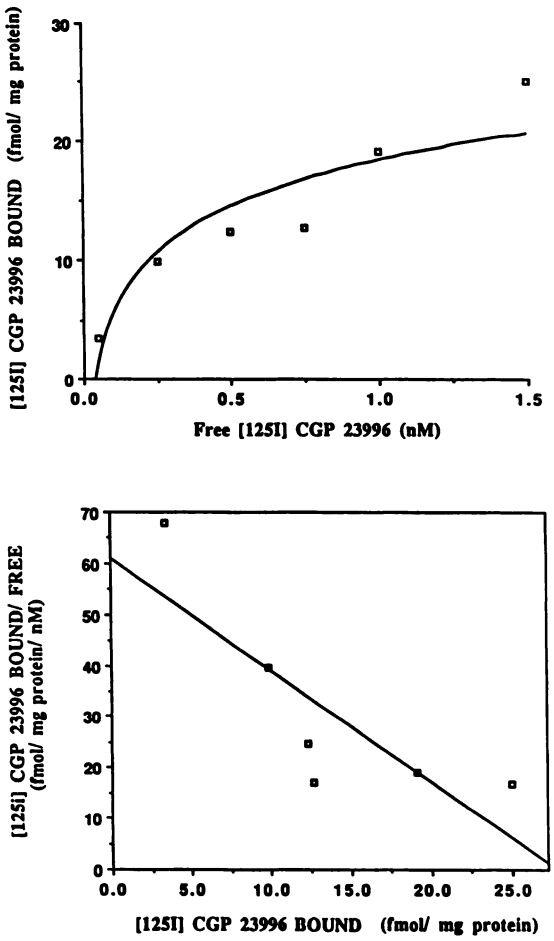


**Fig. 3.** Saturable binding of $^{125}$I-CGP 23996 to SRIF receptors in rat neocortical membranes. Rat neocortical membranes were incubated for 90 min at 25° with varying concentrations of $^{125}$I-CGP 23996, in the presence or absence of 100 nM SRIF, to determine specific binding (□). *Upper*, a representative saturation isotherm of specific $^{125}$I-CGP 23996 binding; *lower*, linearization of this saturation isotherm. Analysis of the results of three different experiments revealed that $^{125}$I-CGP 23996 bound to SRIF receptors in neocortical membranes with a $K_D$ of 0.44 ± 0.2 nM and a $B_{max}$ of 27 ± 6 fmol/mg of protein.

activity in neocortical membranes by 29 ± 2% (basal activity = 4.5 pmol of cAMP/6 min/mg of protein; stimulated activity = 11.2 pmol of cAMP/6 min/mg of protein; results are the mean ± standard error of four different experiments) at a concentration of 10 μM. Similarly, 10 μM MK 678 and CGP 23996 also inhibited forskolin-stimulated adenylate cyclase activity by 26 ± 3% and 28 ± 2%, respectively.

In previous studies, we reported that SRIF inhibits voltage-dependent $Ca^{2+}$ currents in rat neocortical neurons in culture (15). SRIF reduced a high voltage-activated $Ca^{2+}$ current by 30–40% in approximately 60% of the cells tested, with an $IC_{50}$ value of 15 nM (15). SRIF did not affect the low voltage-activated $Ca^{2+}$ current in these cells. Both MK 678 and CGP 23996 also reduced a high voltage-activated $Ca^{2+}$ current in rat neocortical neurons (Fig. 5), while not affecting the low voltage-activated $Ca^{2+}$ current (data not shown). In 10 cells tested, MK 678 reduced the high voltage-activated $Ca^{2+}$ current in six cells by an average of 36 ± 1%. The other four cells tested did not respond to MK 678. In 15 other cells tested, CGP 23996 reduced the $Ca^{2+}$ current in 10 cells by an average of 27 ± 2% (Fig. 5). The other five cells tested did not respond to CGP 23996.

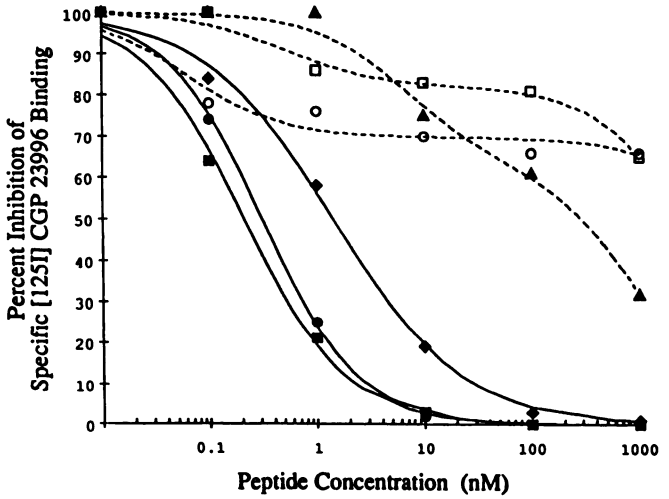


**Fig. 4.** Specificity of $^{125}$I-CGP 23996 binding to SRIF receptors in rat neocortical cell membranes. $^{125}$I-CGP 23996 (0.5 nM) binding to membranes of rat neocortical cells was inhibited by varying concentrations of SRIF (●), SRIF-28 (■), SMS-201-995 (▲), MK 678 (○), MK 363-301 (□), and CGP 23996 (◆). These are the averaged results of at least three different experiments.

In previous studies, we reported that SRIF potentiates $I_K$ in rat neocortical neurons (14, 28). This effect is reversible, of high potency, specific, and observed in approximately 65% of the cells tested (14, 28). MK 678 also potentiated $I_K$ in rat neocortical neurons in a reversible manner (Fig. 6). In 14 cells tested, MK 678 potentiated $I_K$ in eight cells by an average of 26 ± 2%. The other six cells tested did not respond to MK 678. In contrast to MK 678, CGP 23996 did not alter $I_K$ in rat neocortical neurons (Fig. 6). In 14 cells tested, none responded to CGP 23996 with changes in $I_K$. In those cells that did not respond to CGP 23996, MK 678 increased $I_K$ in seven cells by an average of 28 ± 3% (Fig. 6). The other seven cells did not respond to MK 678.

## Discussion

The findings of the present study show that rat cortical cells in culture express multiple SRIF receptor subtypes. This finding is consistent with the results of a number of previous studies showing that pharmacologically and biochemically distinct SRIF receptors are expressed in brain (14, 18–22, 32). Two SRIF receptor subtypes were identified in neocortical cells using ligand binding techniques. They express pharmacological specificities similar to those expressed in adult rat brain. These receptor subtypes were detected using the radioligands $^{125}$I-MK 678 and $^{125}$I-CGP 23996. $^{125}$I-MK 678 bound with high affinity and specificity to a single population of brain and rat neocortical SRIF receptors. These SRIF receptors expressed high affinity for a variety of newly developed SRIF agonists, including CGP 23996. They have similar pharmacological characteristics as the SRIF receptors expressed in pituitary, which mediate the well established function of SRIF to inhibit growth hormone release (21). $^{125}$I-CGP 23996 bound to at least two subtypes of SRIF receptors in rat brain and neocortical cell membranes. One site expresses high affinity for SRIF and MK 678 and is the same site as that detected by $^{125}$I-MK 678. Another site detected by $^{125}$I-CGP 23996 in these cells, as well as in rat brain, expresses high affinity for SRIF and SRIF-28 but is insensitive to MK 678. This SRIF receptor is pharma-

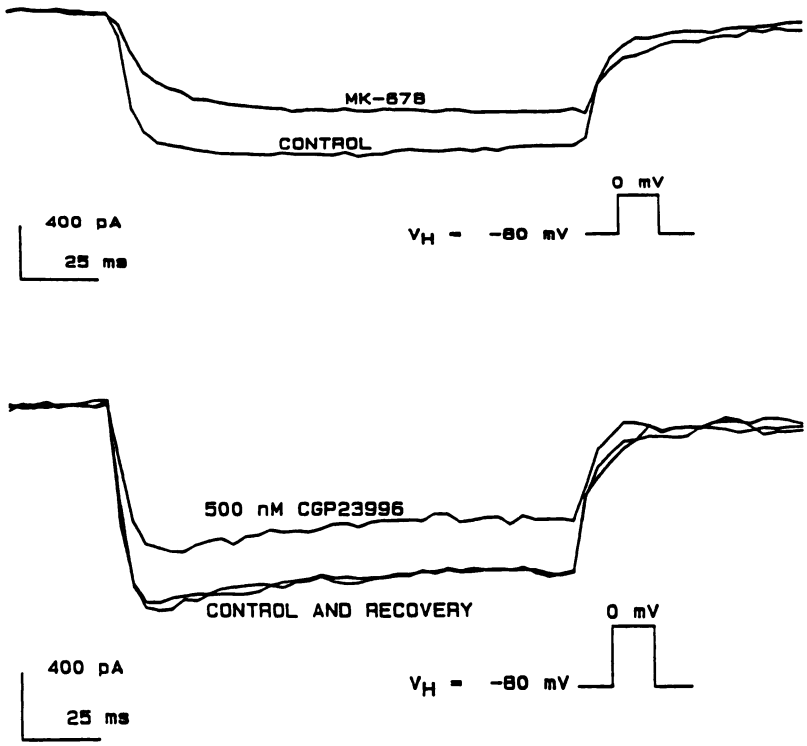


**Fig. 5.** Inhibition of voltage-dependent $Ca^{2+}$ conductance by MK 678 and CGP 23996 in rat neocortical neurons in culture. The effects of 500 nM MK 678 (*upper*) or 500 nM CGP 23996 (*lower*) on the high voltage-activated $Ca^{2+}$ current in neocortical neurons in culture were examined. These are representative traces of the effect of these peptides on the $Ca^{2+}$ currents in different neurons tested.

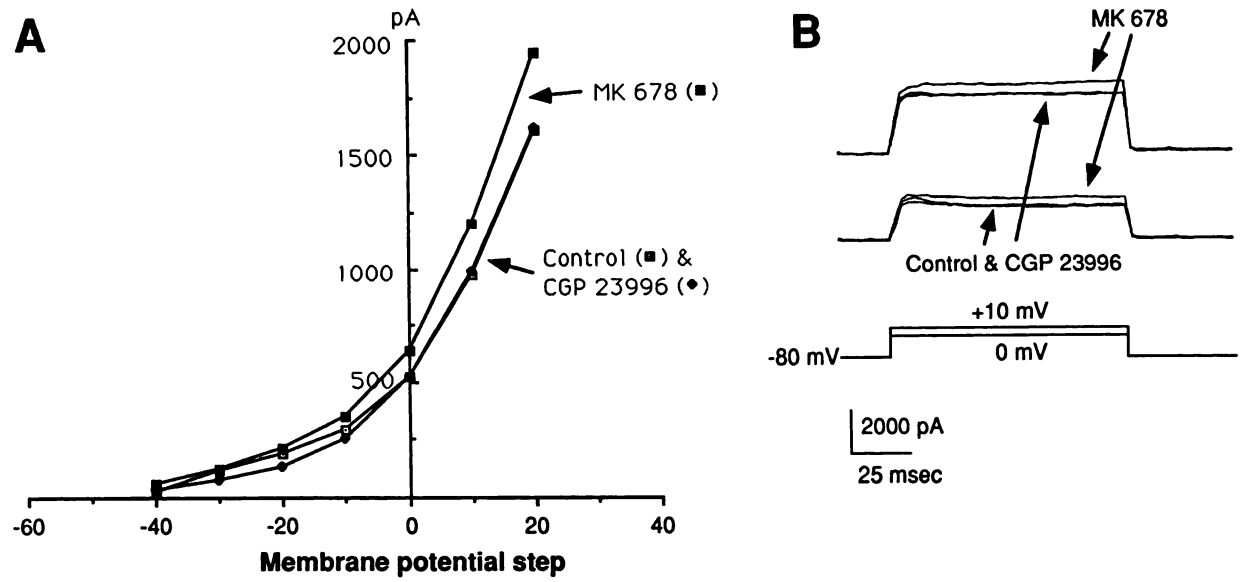


**Fig. 6.** Selective potentiation of $I_K$ by MK 678 in rat neocortical cells in culture. A, Representative *I-V* curve for voltage-dependent $K^+$ currents evoked in the sequential presence of 500 nM CGP 23996 (◆), which is almost superimposable on the control *I-V* curve, and 500 nM MK 678 (■), which shows increased currents at steps more positive than 0 mV. B, Examples of currents evoked by steps from −80 mV to 0 mV (*middle*) and +10 mV (*upper*) from the same neuron sequentially perfused with control bath solution, 500 nM CGP 23996 (superimposed on control), and 500 nM MK 678 (which induced an increase in $I_K$).

cologically distinct from the receptor labeled by $^{125}$I-MK 678 and is not expressed in rat pituitary (21).

The SRIF receptor subtypes detected by $^{125}$I-MK 678 and $^{125}$I-CGP 23996 have similarities to those previously reported by Tran *et al.* (18). These investigators reported that the synthetic analog SMS-201-995 was able to distinguish two SRIF receptors in brain, whereas pituitary expressed only one SRIF receptor. However, SMS-201-995 cannot be used to investigate functions of brain SRIF receptor subtypes, because of its cross-reactivity with opiate receptors (33). In contrast, MK 678 and CGP 23996 are highly specific for brain SRIF receptors and are useful in identifying the functional role of central receptors responsive to SRIF (21, 22).

The SRIF receptors with high affinity for MK 678 and CGP 23996 mediate inhibition of adenylate cyclase activity and modulation of $Ca^{2+}$ currents in neocortical neurons by SRIF. This was shown by the equal effectiveness of SRIF, MK 678, and CGP 23996 to inhibit both responses.

Responses mediated by the SRIF receptor with high affinity for SRIF and CGP 23996 but no affinity for MK 678 would be

expected to be evoked by SRIF and CGP 23996 but not by MK 678. However, no functional response was detected for this receptor in neocortical cells, because every response elicited by CGP 23996 was also induced by MK 678.

Both SRIF and MK 678 potentiated $I_K$ in rat neocortical cells, whereas CGP 23996 was without effect. The concentrations of CGP 23996 used in these studies maximally displaced all $^{125}$I-CGP 23996 and $^{125}$I-MK 678 binding to SRIF receptors and induced the same effects on adenylate cyclase activity and $Ca^{2+}$ currents as similar concentrations of SRIF and MK 678, suggesting that, if receptors responsive to CGP 23996 were coupled to $K^+$ channels in these cells, they should have been activated by the concentrations of CGP 23996 used in these studies. The inability of high concentrations of CGP 23996 to potentiate $I_K$ would suggest that receptors detected in our ligand binding studies may not be involved in mediating this response. These findings suggest that, potentially, a novel receptor can be activated by SRIF and MK 678 that has low or no affinity for CGP 23996 and mediates the effects of SRIF on $I_K$. Alternatively, the GTP-binding proteins coupling SRIF receptors to $K^+$ channels in these cells may be different from those coupling the same receptors to the catalytic subunit of adenylate cyclase or $Ca^{2+}$ channels. Those GTP-binding proteins may induce a conformation in the SRIF receptor such that it does not recognize CGP 23996 or that CGP 23996 can bind to the receptor but not induce a functional response.

Previous studies have suggested that the receptor mediating the potentiation of $I_K$ by SRIF is different from the one mediating the inhibition of the high voltage-activated $Ca^{2+}$ current and adenylate cyclase activity in neocortical cells by SRIF (14, 15, 28). The facilitation of $I_K$ by SRIF desensitizes (28), whereas the inhibition of the $Ca^{2+}$ current is resistant to desensitization (15). Furthermore, both SRIF and SRIF-28 inhibit the high voltage-activated $Ca^{2+}$ current (15) as well as adenylate cyclase activity (14) in neocortical cells. However, SRIF and SRIF-28 induce opposite effects on $I_K$ (14, 28).

The results of the present study indicate that SRIF receptors with high affinity for MK 678 mediate the effects of SRIF on adenylate cyclase activity and $Ca^{2+}$ and $K^+$ currents. SRIF receptor subtypes coupled to these cellular effector systems can be distinguished by their differing sensitivities to the SRIF agonist CGP 23996. Development of selective agonists and antagonists will be necessary to further identify the functional role of these SRIF receptor subtypes.

**References**


1. Epelbaum, J. Somatostatin in the central nervous system: physiology and pathological modifications. *Prog. Neurobiol.* **27**:63–100 (1986).
2. Rezek, M., V. Havlicek, L. Leybin, C. Pinsky, E. Kroeger, K. Hughes, and H. Friesen. Neostriatal administration of somatostatin: differential effect of small and large doses on behavior and motor control. *Can. J. Physiol. Pharmacol.* **55**:234–242 (1977).
3. Martin-Iversen, M., J. Radke, and S. Vincent. The effects of cysteamine on dopamine-mediated behaviors: evidence for dopamine-somatostatin interactions in the striatum. *Pharmacol. Biochem. Behav.* **24**:1707–1714 (1986).
4. Fink, J., and J. Martin. Behavioral effects of intrastriatal infusions of somatostatin and somatostatin analogs. *Soc. Neurosci. Abstr.* **14**:174 (1988).
5. Haroutunian, V., R. Mantin, G. Campbell, G. Tsuboyama, and K. Davis. Cysteamine-induced depletion of central somatostatin-like immunoreactivity: effects on behavior, learning memory and brain neurochemistry. *Brain. Res.* **403**:234–242 (1987).
6. DeNoble, V., D. Hepler, and R. Barto. Cysteamine-induced depletion of somatostatin produces differential cognitive deficits in rats. *Brain Res.* **482**:42–48 (1989).
7. Fitzgerald, L. W., and C. Dokla. Morris water task impairment and hypoactivity following cysteamine-induced reductions of somatostatin. *Brain Res.* **505**:246–250 (1989).
8. Dodd, J., and J. Kelly. Is somatostatin an excititory transmitter in the hippocampus? *Nature (Lond.)* **273**:674–677 (1978).
9. Olpe, H., V. Balcar, H. Bittiger, H. Rink, and P. Sieber. Central actions of somatostatin. *Eur. J. Pharmacol.* **63**:127–133 (1980).
10. Delfs, J., and M. Dichter. Effects of somatostatin on the mammalian cortical neurons in culture: physiological actions and unusual dose response characteristics. *J. Neurosci.* **3**:1176–1188 (1983).
11. Pittman, Q., and G. Siggins. Somatostatin hyperpolarizes hippocampal pyramidal neurons *in vitro*. *Brain Res.* **121**:402–408 (1981).
12. Inoue, M., S. Nakajima, and Y. Nakajima. Somatostatin induces an inward rectification in rat locus coeruleus neurones through a pertussis toxin-sensitive mechanism. *J. Physiol. (Lond.)* **407**:177–198 (1988).
13. Jacquin, T., J. Champagnat, S. Mandamba, M. Denavit-Saubie, and G. Siggins. Somatostatin depresses excitability in neurons of the solitary tract complex through hyperpolarization and augmentation of $I_m$, a non-inactivating voltage-dependent outward current blocked by muscarinic agonists. *Proc. Natl. Acad. Sci. USA* **85**:948–952 (1988).
14. Wang, H., C. Bogen, T. Reisine, and M. Dichter. Somatostatin-14 and somatostatin-28 induce opposite effects on potassium currents in rat neocortical neurons. *Proc. Natl. Acad. Sci. USA* **86**:9616–9620 (1989).
15. Wang, H., T. Reisine, and M. Dichter. Somatostatin-14 and somatostatin-28 inhibit calcium currents in rat neocortical neurons. *Neuroscience* **38**:335–342 (1990).
16. Ikeda, S., and G. Schofield. Somatostatin blocks a $Ca^{2+}$ current in rat sympathetic ganglion neurons. *J. Physiol. (Lond.)* **409**:221–240 (1989).
17. Chneiweiss, H., P. Bertrand, J. Epelbaum, C. Kordon, J. Glowinski, J. Premont, and A. Enjalbert. Somatostatin receptors on cortical neurons and adenohypophysis: comparison between specific binding and adenylate cyclase inhibition. *Eur. J. Pharmacol.* **138**:249–255 (1987).
18. Tran, V., M. Beal, and J. Martin. Two types of somatostatin receptors differentiated by cyclic somatostatin analogs. *Science (Washington, D. C.)* **228**:492–495 (1985).
19. Reubi, J. C. New specific radioligand for one subpopulation of brain somatostatin receptors. *Life Sci.* **36**:1829–1836 (1985).
20. Thermos, K., H. T. He, H. Wang, N. Margolis, and T. Reisine. Biochemical properties of brain somatostatin receptors. *Neuroscience* **31**:131–141 (1989).
21. Raynor, K., and T. Reisine. Analogues of somatostatin selectively label distinct subtypes of somatostatin receptors in rat brain. *J. Pharmacol. Exp. Ther.* **251**:510–517 (1989).
22. Martin, J. L., M. F. Chesselet, K. Raynor, C. Gonzales, and T. Reisine. Differential distribution of somatostatin receptor subtypes in rat brain revealed by newly developed somatostatin analogs. *Neuroscience* **41**:581–593 (1991).
23. Fukuda, K., T. Kubo, I. Adiba, A. Maeda, M. Mishina, and S. Numa. Molecular distinction between muscarinic acetylcholine receptor subtypes. *Nature (Lond.)* **327**:623–625 (1987).
24. Kebabian, J., and D. Calne. Multiple receptors for dopamine. *Nature (Lond.)* **277**:93–96 (1979).
25. Lefkowitz, R., and M. Caron. Adrenergic receptors. *J. Biol. Chem.* **263**:4993–4996 (1988).
26. Dichter, M. Rat cortical cells in culture: culture methods, cell morphology, electrophysiology and synaptic formation. *Brain Res.* **149**:279–293 (1978).
27. Legido, A., S. Reichlin, M. Dichter, and J. Buchalter. Expression of somatostatin and GABA immunoreactivity in cultures of rat hippocampus. *Peptides* **11**:103–109 (1990).
28. Wang, H., M. Dichter, and T. Reisine. Lack of cross-desensitization of somatostatin-14 and somatostatin-28 receptors coupled to potassium channels in rat neocortical neurons. *Mol. Pharmacol.* **38**:357–361 (1990).
29. Mahy, N., M. Woolkalis, K. Thermos, K. Carlson, D. Manning, and T. Reisine. Pertussis toxin modifies the characteristics of both the inhibitory GTP-binding protein and the somatostatin receptor in anterior pituitary tumor cells. *J. Pharmacol. Exp. Ther.* **246**:779–785 (1988).
30. Solomon, Y., C. Londos, and M. Rodbell. A highly sensitive adenylate cyclase assay. *Anal. Biochem.* **58**:541–548 (1974).
31. Pless, J., W. Bauer, U. Briner, W. Doepfner, P. Marbach, R. Maurer, T. Petcher, J. Reubi, and P. Vonderscher. Chemistry and pharmacology of SMS 201-995, a long-acting octapeptide analogue of somatostatin. *Scand. J. Gastroenterol.* **21**:54–64 (1986).
32. Reisine, T., K. Raynor, H. L. Wang, and M. Dichter. Subtypes of somatostatin receptors can be differentially regulated and can couple to distinct cellular effector systems. *Neurosci. Abstr.* **16**:84 (1990).
33. Maurer, R., B. Gaehwiler, H. Buescher, R. Hill, and D. Roemer. Opiate antagonistic properties of an octapeptide somatostatin analog. *Proc. Natl. Acad. Sci. USA* **79**:4815–4817 (1982).


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