in D_2 affinity, although these varied significantly with the nature of the *N*-alkyl substituent. Thus, with 2-fluoro substitution, there was an important gain in D_2 affinity (68 times) and selectivity (138 times) for 2-F-NPA over that of NPA (Table I), whereas 2-fluoroapomorphine was only ca. 50% more potent than apomorphine itself in competing in a radioreceptor binding assay at D_2 sites;[4] 2-hydroxy substitution of apomorphine and NPA had a more similar D_2 affinity enhancing effect, although this was somewhat greater with apomorphine (29-fold[3]) than with NPA (15-fold; Table I). It is not clear whether lipophilicity or bulk of the *N*-alkyl substituent in the B ring contributes critically to the effect of substituting an electronegative group in the 2-position of aporphine A ring, but it does appear that the nature of the *N*-alkyl substituent contributes to the effects obtained with some 2-substituents.

Additional information concerning the structure–activity relations involving 2-substitution of NPA includes the much smaller effect of adding a Br than a F atom on increasing D_2 affinity and selectivity (which were more than an order of magnitude lower with 2-Br-NPA than with 2-F-NPA), as well as a somewhat smaller effect on decreasing D_1 affinity (Table I). While the differences between halogen-substituted NPAs may reflect the greater bulk of the Br vs F atom (possibly leading to a less favorable steric interaction at DA receptor surfaces), an alternative possibility is that Br may participate less well than F in hydrogen bonding with the receptor surface. An altogether different effect was found with 2-NH_2 substitution, which markedly diminished D_1 affinity of NPA and tended also to reduce D_2 affinity somewhat with NPA (Table I) and apomorphine[4] by an uncertain mechanism. Regarding D_1 sites, all 2-substituents tested consistently decreased D_1 affinity, possibly reflecting steric interference at the D_1 receptor surface, although this effect usually was relatively small except with 2-NH_2-NPA, as mentioned above (Table I). As was predicted by earlier studies of aporphines with occluded or missing hydroxy groups in the D ring, occlusion of the catechol moiety of 2-F- and 2-NH_2-NPA with a 10,11-methylenedioxy (MDO) bridge, markedly reduced D_2 receptor affinity while having little apparent additional effect on D_1 affinity (Table I); this observation confirms the importance of a free hydroxy group, especially in the 11-position on the aporphine D ring analogous to the *m*-OH in DA, for high D_2 affinity in aporphines.[9,10,11]

The present results, based on the preparation and DA-receptor affinity testing of a series of novel 2-substituted *N*-*n*-propylnorapomorphine (NPA) derivatives, indicate that affinity at D_1 sites was reduced, but only moderately and without a clear relationship on the type of substituent, except that a 2-NH_2 substituent markedly reduced D_1 affinity. More importantly, however, D_2 affinity usually was enhanced by 2-substitution of NPAs, and this effect was particularly striking with a 2-F substituent. Comparison of these results with *N*-*n*-propylaporphines (NPAs) to previous results with 2-*N*-methylaporphines (apomorphines) indicated, further, that the enhancement of D_2 affinity was influenced appreciably, though somewhat inconsistently, by the *N*-alkyl side chain.

A particularly important conclusion is that *R*-(−)-2-F-NPA had the highest D_2 binding affinity (IC_{50} = 71 pM; K_i = 12 pM) and D_2 selectivity (nearly 60 000 by D_1/D_2 ratio of K_i values) of any ligand yet described (Table I), including a series of aminotetralines, ergolines, and phenethylamines which were evaluated in another report.[12] The high affinity of 2-F-NPA led to the prediction that it would have high potency in a behavioral test of central DA agonist activity (induction of stereotyped gnawing in the rat), and it was found to be about ten-times more potent than NPA.[12] In addition to the potential experimental or medicinal interest in such a potent and selective, centrally neuropharmacologically active D_2 agonist as R-(−)-2-F-NPA, it should also be pointed out that this congener, R-(−)-2-NH_2-NPA, could serve as a precursor for the preparation of ^{18}F-labeled R-(−)-2-F-NPA, a potential imaging agent for positron emission tomography (PET) studies of agonist-labeled DA receptors in vivo.

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Expedient Synthesis and Biochemical Properties of an [^{125}I]-Labeled Analogue of Glyburide, a Radioligand for ATP-Inhibited Potassium Channels

Potassium (K) channels are ubiquitous and play critical and complex roles in the control of membrane potential in most excitable cells. As a consequence, a variety of physiological processes such as neurotransmitter release, electrical conduction in the heart, and insulin secretion are

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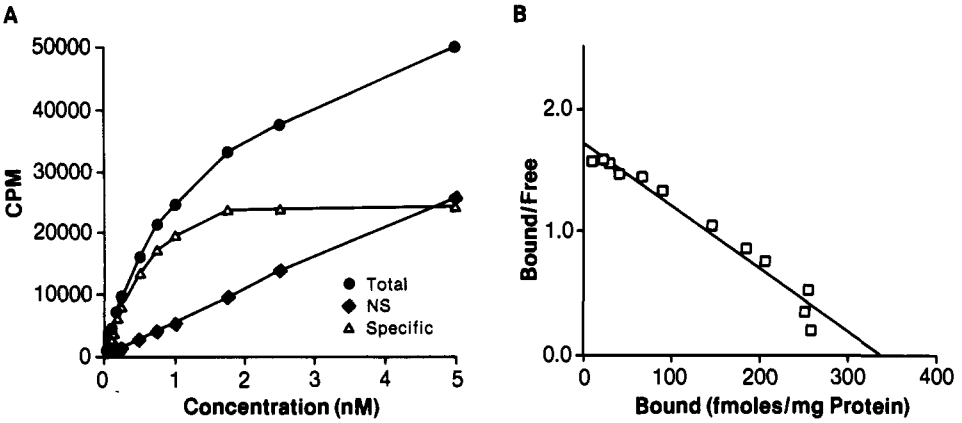

Figure 1. Saturation isotherm (panel A) and Scatchard analysis (panel B) of [^{125}I]-1 binding to rat brain membranes. Male Sprague-Dawley rat brains (Pel-Freeze, Rogers, AR) were thawed and homogenized in 50 mM TRIS buffer (pH 7.4 at 4 °C) with use of a Brinkman Polytron. After an initial spin at 100*g*, membranes were pelleted by centrifugation at 30000*g*, resuspended in buffer and pelleted again. Incubations were performed in a 0.2-mL final volume containing 20 mM HEPES (pH 7.4), 10–5000 pM [^{125}I]-1, and 50 μg of brain protein. Nonspecific binding was determined by incubating tissue in the additional presence of 1 μM glyburide. Following a 1-h incubation period at 4 °C, the binding reaction was terminated by rapid filtration over Whatman GF/C filters which were presoaked for 1 h in 1% polyethyleneimine (Sigma). Binding was assessed by γ counting and the data analyzed by using Lundon software (Chagrin Fall, OH). Data represent the mean of two experiments performed in duplicate.

modulated in part by appropriate functioning of K channels.[1]

A number of K channel subtypes have been identified, and the ATP-inhibited K channel has engendered considerable interest recently. In pancreatic β-cells, this ligand-gated K channel subtype appears to be the site of action of the sulfonylurea-derived antidiabetic drugs, including glyburide[2] and glipizide.[3,4] Moreover, the ATP-inhibited K channel in vascular smooth muscle has been implicated as the probable site of action of a new class of vasodilators, including cromakalim and pinacidil, which function by opening K channels and thereby producing hyperpolarization of the cells.[5] Finally, this channel may play a role in cellular damage resulting from various forms of cerebral ischemia including stroke and trauma.[6-8]

The development of radioligands that bind avidly and selectively to subtypes of K channels is an important goal for K channel research, and two such probes have been described: radioiodinated charybdotoxin labels high-conductance, calcium-activated K channels[9] and [^{3}H]glyburide labels ATP-inhibited K channels.[4,10] The availability of these ligands should permit a variety of useful biochemical experiments.

Because ATP-inhibited K channels are present in vascular smooth muscle and brain tissue in low numbers (ca. 50–300 fmol/mg protein), a number of experiments we envisaged required an easily synthesized sulfonylurea-derived radioligand with a specific activity much greater than can be obtained with tritium-labeled probes (typically 20–100 Ci/mmol). Such an agent would be particularly useful for autoradiographic experiments to reduce the exposure times (months) required for these tritium-based radioligands; a high specific activity radioligand would also be of use for a variety of additional molecular biology and biochemical applications. The isotope ^{125}I appeared to be a useful radionuclide for our purposes because of its high specific activity (2100 Ci/mmol) and the ability to readily incorporate it into organic molecules. In this communication, we report an expedient synthesis of the radioiodinated isostere of glyburide, *N*-[2-[4-[[[(cycohexylamino)carbonyl]amino]sulfonyl]phenyl]ethyl]-5-[^{125}I]-iodo-2-methoxybenzamide (LY285110, compound 1), and its use to specifically label ATP-inhibited K channels in rat brain. Other workers have recently demonstrated that another iodinated glyburide analogue may be used as a radioligand and photoaffinity label for ATP-inhibited K channels in a hamster pancreatic cell line.[11]

Synthesis. Commercially available 5-iodosalicylic acid was converted to 5-iodo-2-methoxybenzoic acid by permethylation with dimethyl sulfate and potassium carbonate, followed by sodium hydroxide hydrolysis of the resulting ester (Scheme I). The acid chloride was generated with thionyl chloride and then reacted with 4-(2-aminoethyl)benzenesulfonate in 2 N sodium hydroxide to provide **5**. Reaction of **5** with potassium carbonate and cyclohexyl isocyanate in acetone provided unlabeled **1**.[12]

Because of recent advances in tin chemistry as applied to synthesis of radiopharmaceuticals,[13] compound 1 served as the starting material in the synthesis of the radionuclide-bearing version: reaction of 1 with hexamethylditin and tetrakis(triphenylphosphine)palladium(0) in refluxing dioxane for 3 h yielded **6**. Exposure of this arylstannane

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(2) Glyburide is also known as glibenclamide, the accepted nonproprietary name in Europe. The systematic chemical name is *N*-[2-[4-[[[(cyclohexylamino)carbonyl]amino]sulfonyl]-phenyl]ethyl]-5-chloro-2-methoxybenzamide.
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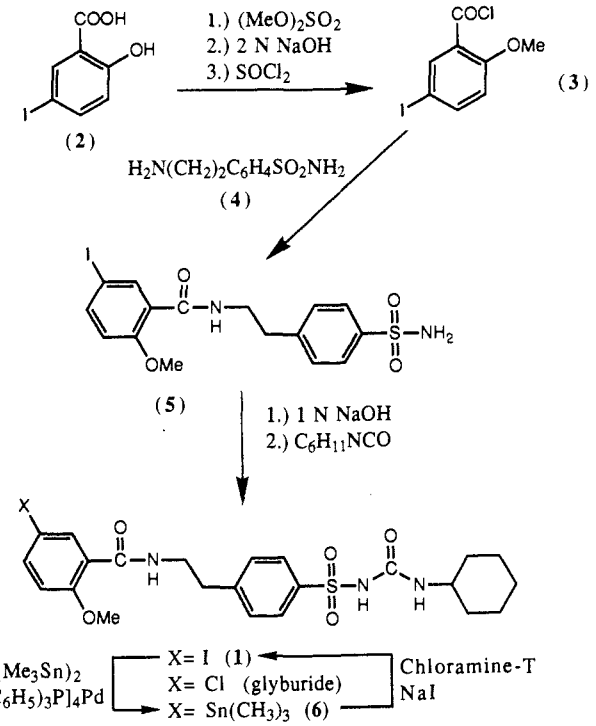
Scheme I



derivative to chloramine-T and sodium [^{125}I]iodide in methanol, followed by HPLC purification produced [^{125}I]-1.[14] The product was pure by HPLC and comigrated on TLC plates with authentic unlabeled material; the radiochemical yield based on inorganic iodide was 78%. Importantly, the tin derivative **6** is stable, and [^{125}I]-1 can be readily generated and purified from this storable precursor.

Biochemistry. Binding of [^{125}I]-1 was evaluated by using rat brain homogenate preparations and a rapid filtration assay; the methods are summarized in the legend of Figure 1. The binding of [^{125}I]-1 to rat brain membranes was saturable, and the nonspecific binding was low (Figure 1, panel A). Scatchard analysis of the saturation isotherm (panel B) demonstrated a single, homogeneous population of binding sites with a K_d of 195 pM, and a B_{max} of 340 fmol/mg protein. These data are in reasonable agreement with values reported for labeling of K channels in pig cortex microsomes by [^{3}H]glyburide (K_d = 800 pM; B_{max} = 400 fmol/mg protein).[15] Glyburide and unlabeled **1** were potent inhibitors of specific [^{125}I]-1 binding (Figure 2) with K_i values of 608 pM and 2.6 nM, respectively. These data indicate that replacement of the chlorine atom in glyburide with iodine results in an approximately 4-fold decrease in affinity for the ATP-inhibited K channel; nevertheless, **1** still binds with high affinity to this site. Glipizide, another therapeutically useful sulfonylurea antidiabetic drug, was somewhat less potent as an antagonist of [^{125}I]-1 binding, with a K_i value of 8.2 nM. The K channel opener pinacidil inhibited the specific binding of [^{125}I]-1 but at concentrations in excess of 10^{-4} M. The fact

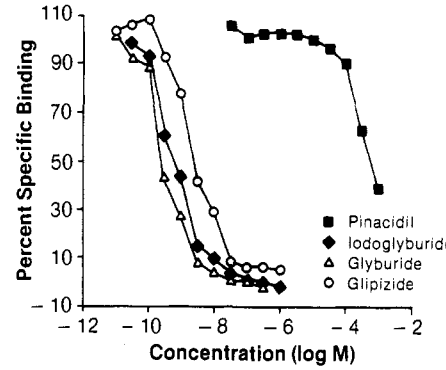


Figure 2. Displacement of [^{125}I]-1 by modulators of ATP-inhibited K channels. Displacement curves were generated by using a 100 pM concentration of [^{125}I]-1 and various concentrations of the inhibitors as indicated in the figure. Data represent the mean of two experiments performed in duplicate.

that relatively high concentrations of K channel openers are required to specifically inhibit binding of [^{3}H]glyburide compared to those required for pharmacological effects has been previously noted,[4] and this may relate to a variety of factors, including complex allosteric interactions among these ligands.[16]

Conclusion

In this communication we have described an efficient synthesis of an [^{125}I]-labeled analogue of glyburide that binds avidly and specifically to sulfonylurea receptors in the brain. The specific binding of [^{125}I]-1 to rat brain membranes could be inhibited in a concentration-dependent manner by sulfonylurea antidiabetic drugs which function as antagonists of ATP-dependent K channels. Because of its ease of preparation, high specific binding, and high specific activity, this compound appears to be a useful biochemical probe of ATP-inhibited K channels. These characteristics make this radiolabeled antagonist a particularly useful tool to study ATP-inhibited K channels where they are present in low density (e.g. vascular smooth muscle and the CNS) and for autoradiographic studies; in preliminary experiments[17] we have obtained quantitative rat brain autoradiograms with 24–48-h exposure of brain slices labeled with [^{125}I]-1. In addition to the advantage of rapid exposure time, the low-energy γ emissions from [^{125}I]-1 allow autoradiographic quantification of K channels in white matter where lower energy β-emissions from tritium are quenched.[18]

Acknowledgment. We thank Denise A. Hunter for preparation of the manuscript and Dr. Mitchell I. Steinberg for stimulating discussions and encouragement.

(14) 6 (10 nmol) was dissolved in 25 μL of methanol, and 1 mCi of sodium [^{125}I]iodide (2100 Ci/mmol) was added, followed by the addition of 5 μL of chloramine-T (5 mg/mL in 200 mM phosphate buffer, pH 7.5). The reaction was allowed to proceed for 4 min and was then purified on an ODS-3 reverse-phase column utilizing a mobile phase of 60% methanol and 40% ammonium acetate (0.1 M); flow rate was 1 mL/min. The radioiodinated material, **1**, elutes at 9–10 min under these conditions. The specific activity of the product was approximately 2100 Ci/mmol, and the radiochemical yield was 78%.

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