4. Björkstén B, Ray C and Quie PG, Inhibition of human neutrophil chemotaxis and chemoluminescence by amphotericin B. *Infect Immunol* **14**: 315–317, 1976.
5. Marmer DJ, Fields BT, France GL and Steele RW, Ketoconazole, amphotericin B and amphotericin B methyl ester: comparative *in vitro* and *in vivo* toxicological effects on neutrophil function. *Antimicrob Agents Chemother* **20**: 660–665, 1981.
6. Lohr KM and Snyderman R, Amphotericin B alters the affinity of the oligopeptide chemotactic factor receptor on human polymorphonuclear leukocytes. *J Immunol* **129**: 1594–1599, 1982.
7. Boxer LA, Ingraham LM, Allen J, Oseas RS and Baehner RC, Amphotericin B promotes leukocyte aggregation of nylon-wool fiber-treated polymorphonuclear leukocytes. *Blood* **58**: 518–523, 1981.
8. Ferrante A and Thong YH, Simultaneous preparation of mononuclear and polymorphonuclear leukocytes from horse blood on Ficoll-Hypaque medium. *J Immunol Methods* **34**: 279–285, 1980.
9. Trush MA, Wilson ME and Van Dyke K, The generation of chemiluminescence by phagocytic cells. *Methods Enzymol* **57**: 462–494, 1978.
10. Whitin JC, Clarks RA, Simons ER and Cohen HJ, Effects of myeloperoxidase system on fluorescent probes of granulocyte membrane potential. *J Biol Chem* **256**: 8904–8906, 1981.
11. Seligmann BE and Gallin JI, Comparison of indirect probes of membrane potential utilized in studies of human neutrophils. *J Cell Physiol* **115**: 105–115, 1983.
12. Holian A and Daniele RP, Stimulation of oxygen consumption and superoxide anion production in pulmonary macrophages by *N*-formyl methionyl peptides. *FEBS Lett* **108**: 47–50, 1979.
13. Vertut-Croquin A, Bolard J and Gary-Bobo CM, Transfer of amphotericin B gel state vesicles to mycoplasma cells: biphasic action on potassium transport and permeability. *Antimicrob Agents Chemother* **28**: 167–171, 1985.
14. Roberts J and Quastel JH, Particle uptake by polymorphonuclear leucocytes and Ehrlich ascites-carcinoma cells. *Biochem J* **89**: 150–156, 1963.
15. Orr W and Ward PA, Quantitation of leukotaxis in agarose by three different methods. *J Immunol Methods* **20**: 95–107, 1978.
16. Newburger PE, Chovaniec ME and Cohen HJ, Activity and activation of the granulocyte superoxide-generating system. *Blood* **55**: 85–92, 1980.
17. Wilson M, Trush MA and Van Dyke K, Effect of ionophore A23187 on chemiluminescence by human granulocytes. *Fed Proc* **36**: 1071, 1977.
18. Seligmann BE and Gallin JI, Use of lipophilic probes of membrane potential to assess human neutrophil activation. *J Clin Invest* **66**: 493–503, 1980.
19. Majander A and Wilkström M, The plasma membrane potential of human neutrophils. Role of ion channels and the sodium/potassium pump. *Biochim Biophys Acta* **980**: 139–145, 1989.
20. Schneider E, Haest CWM, Plasa G and Deuticke B, Bactericidal cytotoxins, amphotericin B and local anesthetic enhance transbilayer mobility of phospholipids in erythrocyte membranes. *Biochim Biophys Acta* **855**: 325–336, 1986.
21. Brajtburg J, Elberg S, Medoff J, Kobayashi G, Schlessinger D and Medoff G, Stimulating, permeabilizing and toxic effects of amphotericin B. *Antimicrob Agents Chemother* **26**: 892–897, 1984.
22. Brajtburg J, Elberg S, Schwartz DR, Vertut-Croquin A, Schlessinger D, Kobayashi GS and Medoff G, Involvement of oxidative damage in erythrocyte lysis induced by amphotericin B. *Antimicrob Agents Chemother* **27**: 172–176, 1985.
23. Weening RS, Roos D, Weemaes CMR, Homan-Müller JWT and Schaik MLJ, Defective initiation of the metabolic stimulation in phagocytizing granulocytes. *J Lab Clin Med* **88**: 757–768, 1976.
24. Harvath L and Anderson BR, Defective initiation of oxidative metabolism in polymorphonuclear leukocytes. *N Engl J Med* **300**: 1130–1135, 1979.

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Inhibition of dopamine synthesis in rat striatal minces: evidence of dopamine autoreceptor supersensitivity to *S*(+)- but not *R*(−)-*N*-*n*-propylnorapomorphine after pretreatment with fluphenazine

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Striatal dopamine (DA*) synthesis can be modulated by DA agonists acting at nerve terminal and other autoreceptors to inhibit the rate-limiting tyrosine hydroxylase (EC 1.14.16.2) step in DA biosynthesis [1]. Through an apparent autoreceptor-mediated mechanism, the apomorphine congener *R*(−)-*N*-*n*-propylnorapomorphine (NPA) potently inhibits DA synthesis in rat striatal minces and synaptosomes [2, 3], as well as in an *in vivo* model [4] measuring accumulation of L-dihydroxyphenylalanine (DOPA) in rat striatum [5]. Both *S*(+)- and *R*(−)-NPA are full agonists at postsynaptic D_2 receptors in rat pituitary gland, where the *R*(−) enantiomer is about 45-fold more potent [6]. At synthesis-modulating striatal D_2 autoreceptors, both isomers also are full agonists but *S*(+)-NPA is nearly equipotent to *R*(−)-NPA [2]. We have proposed [2] that *S*(+)-NPA is a relatively selective full agonist at presynaptic D_2 autoreceptors mediating inhibition of DA synthesis, where its efficacy may depend on a relatively high abundance or "reserve" of striatal D_2 autoreceptors [7], while *R*(−)-NPA is a nonselective full agonist at both pre- and postsynaptic D_2 receptors.

* Abbreviations: DA, dopamine; NPA, *N*-*n*-propylnorapomorphine; DOPA, dihydroxyphenylalanine; and GBL, γ-butyrolactone.

It is well established that postsynaptic D_2 receptors upregulate and become supersensitive following repeated treatment with typical neuroleptics or other agents which remove DA or block its synthesis [8]. Thus, administration of neuroleptics, reserpine, or α-methyltyrosine for 21 days results in an approximate 2-fold increase in the potency of apomorphine to elicit stereotyped gnawing behavior in rats [8], and similar neuroleptic treatment can moderately increase the total density (maximum radioligand binding) of D_2 receptors in rat striatal tissue by about 10–30% [9, 10]. Suggestive evidence of supersensitivity of *autoreceptors* mediating DA synthesis has been obtained using low doses of DA agonists, such as *R*(−)-apomorphine, which are about 20% more potent in reducing striatal DA turnover in rat brain *in vivo* after repeated neuroleptic treatment [11]. Further evidence of DA autoreceptor supersensitivity is provided by an approximately 25% increase in the potency of apomorphine, after repeated haloperidol treatment, in reducing striatal accumulation of DOPA in rats treated with a decarboxylase inhibitor and γ-butyrolactone GBL [12]. Additionally, *in vitro* inhibition of DA release from rabbit striatal slices by apomorphine, presumably also by activation of presynaptic autoreceptors, was enhanced by about 50% following repeated haloperidol treatment [13]. In an effort to probe further, the mechanisms of presynaptic receptor-mediated modulation of DA synthesis *in vitro*, we examined the effects of acute and repeated administration of fluphenazine on inhibition of tyrosine hydroxylase by the *S*(+) and *R*(−) enantiomers of NPA in minces of corpus striatum from rat forebrain; this assay was used previously to evaluate D_2 autoreceptor-mediated effects of the NPA isomers with normal brain tissue [2, 3].

Materials and Methods

Chemicals. L-[1-^{14}C]Tyrosine (48.6 mCi/mmol) was purchased from DuPont-NEN, Inc. (Boston, MA). *S*(+)- and *R*(−)-*N*-*n*-propylnorapomorphine hydrochloride were donated by Research Biochemicals Inc. (Natick, MA), and fluphenazine hydrochloride by the E. R. Squibb Corp. (New Brunswick, NJ). Other chemicals were obtained from Fisher Scientific (Pittsburgh, PA) or the Sigma Chemical Co. (St. Louis, MO) in the highest available purity.

Animals. Young adult (250–300 g) male Sprague–Dawley albino rats (Charles River Laboratories, Wilmington, MA), given free access to food and water, received fluphenazine-HCl [dissolved in a drop of 80% (w/v) lactic acid and diluted in water] intraperitoneally (i.p.) at 1 mg/kg (2.1 μmol/kg) or the vehicle, once daily for 10 days, or at 10 mg/kg one time only. Animals were killed 4 days after the last injection to allow elimination of fluphenazine from brain [14].

Tyrosine hydroxylase activity in striatal minces. Activity of tyrosine hydroxylase as a measure of DA synthesis was assessed by measuring the formation of $^{14}CO_2$ evolved during the decarboxylation of DOPA to form DA, starting from L-[1-^{14}C]tyrosine, as described elsewhere [2]. Briefly, corpus striata of rats killed by decapitation were dissected on ice, minced in 0.32 M sucrose, and diluted to 25 mg/mL with ice-cold, oxygenated, modified [2] Krebs–Ringer physiological assay buffer (pH 7.0). Of this suspension, 300 μL (*ca.* 7.5 mg tissue; the average amount of protein per assay tube was 1.22 ± 0.03 mg/mL (mean ± SEM, N = 10) using the bicinchoninic acid assay method [15]) were added to test tubes containing NPA at 0 or 0.2 to 10 μM, preincubated for 5 min at 37°, and reacted with substrate (0.25 μCi of [^{14}C]tyrosine at 20 μM) in a total assay of 0.5 mL for 30 min. The reaction was terminated and $^{14}CO_2$ was liberated with perchloric acid, trapped on phenethylamine-saturated filter paper, and counted by scintillation spectrometry. Values of ^{14}C cpm/30 min/assay tube, less a blank value (with tissue omitted, usually ≤10% of control basal cpm), were determined. Linear relationships were obtained for the amount of normal tissue and length of incubation versus production of $^{14}CO_2$. Typically, three replicates were run for each condition, and each experiment was replicated independently at least once (final N ≥ 6 per point), with data (as percentage of corresponding fluphenazine-treated or untreated basal control values) pooled and expressed as means ± SEM. Values of drug potency as IC_{50} (±SEM) were obtained using the ALLFIT program adapted to a microcomputer [2–4, 16].

Results and Discussion

Tyrosine hydroxylase activity was strongly inhibited by increasing concentrations of *S*(+)-NPA (Fig. 1). The IC_{50} (±SEM) in striatal minces from animals receiving vehicle only was 1.04 ± 0.12 μM, in close agreement with previous results using untreated rats (1.00 ± 0.10 μM) [2]. In animals given the *chronic* fluphenazine pretreatment, the curve was shifted significantly to the left (Fig. 1) by 30% (IC_{50} = 0.73 ± 0.07 μM; $t = 2.23$ [10 df], $P < 0.05$; also by two-way ANOVA, the effect of this treatment was highly significant: $F_{1,47} = 11.7$, $P = 0.0013$), whereas in rats given the fluphenazine acutely, the inhibition of tyrosine hydroxylase by *S*(+)-NPA (IC_{50} = 0.90 ± 0.07 μM; data not shown) did not differ significantly ($P > 0.10$, Student's *t*-test) from rats given vehicle only. Interestingly, the inhibition of tyrosine hydroxylase activity by *R*(−)-NPA at 0.5 μM (*ca.* IC_{50}) (48.7 ± 1.9 and 47.7 ± 3.3% of no-NPA control in chronic fluphenazine-pretreated and control tissues, respectively) and at 1.0 μM (42.5 ± 2.5 and 43.0 ± 2.3%) did not change significantly.

The moderately increased sensitivity of tyrosine hydroxylase to inhibition by *S*(+)-NPA suggests that the function of autoreceptors mediating inhibition of DA synthesis increased somewhat in response to sustained, but not acute DA receptor blockade by repeated fluphenazine treatment. The mechanisms involved may include increased

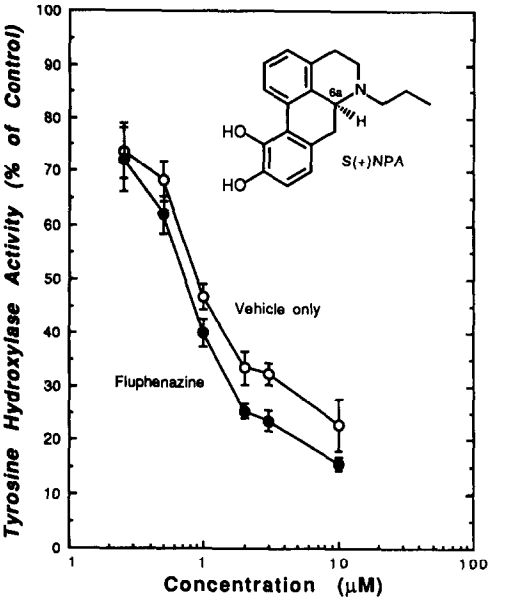

Fig. 1. Concentration–response curves for inhibition by *S*(+)-NPA (chemical structure in inset) of tyrosine hydroxylase activity in minced rat corpus striatum at 4 days after ten daily injections of fluphenazine (1 mg/kg, i.p.) or a control vehicle. Data are means ± SEM of six determinations at each concentration. Control tyrosine hydroxylase activity = 2.70 ± 0.19 pmol $^{14}CO_2$/30 min/mg tissue.

receptor number, more effective coupling of autoreceptors to second messenger systems (perhaps including G_i proteins and adenylate cyclase), or a combination of such changes. The relatively selective full agonist properties of *S*(+)-NPA at the D_2 autoreceptor may depend on the high presynaptic receptor reserve found in rat striatum [2, 7]; despite this relatively large presynaptic receptor reserve, a functionally active pool of D_2 autoreceptors apparently can be increased to some extent in response to repeated pretreatment with a DA antagonist [10–12] (Fig. 1). In contrast, *R*(−)-NPA, a potent and nonselective full agonist at pre- and postsynaptic D_2 receptors [2, 6], failed to show a change in its autoreceptor effect; if it maximally inhibits tyrosine hydroxylase by occupying only a fraction of available autoreceptors, then a moderate change in autoreceptor abundance or sensitivity may have no observable effect on its ability to inhibit DA synthesis.

The 30% increase in potency of *S*(+)-NPA in this study is similar to the approximately 25% increase in potency for *R*(−)-apomorphine to reduce *in vivo* DOPA accumulation in rats given repeated haloperidol treatment [12], and somewhat less than an approximately 50% increase in potency of apomorphine to inhibit DA release in rabbit striatal slices after chronic haloperidol pretreatment [13]. Since modulation of DA synthesis and release may be mediated by different autoreceptor mechanisms [1], these may adapt differently to specific DA antagonists, or vary among animal species, and so contribute to various degrees of apparent autoreceptor supersensitization found in these several experiments. Nevertheless, none of these demonstrations of apparent autoreceptor supersensitivity is as large as the *ca.* 2-fold increases in potency of apomorphine to induce stereotyped behaviors in rats, presumably by stimulating supersensitive *postsynaptic* DA receptors [8]. It may be that a higher receptor reserve at presynaptic than postsynaptic D_2 receptors [7] limits the potential for autoreceptor supersensitization.

Interestingly, the *basal* tyrosine hydroxylase activity in chronic fluphenazine-treated animals was consistently about 25% lower than that observed in untreated animals (100% condition = 2.00 ± 0.18 vs 2.70 ± 0.19 pmol $^{14}CO_2$/30 min/mg wet wt tissue, respectively; N = 6). This finding accords with a previous observation that, in mice given haloperidol chronically, tolerance developed to the initial DA synthesis-increasing effect of the neuroleptic, and after withdrawal from haloperidol there was an approximately 25% lower rate of striatal DA synthesis *in vivo* compared to untreated animals—possibly as a reflection of autoreceptor supersensitivity [17].

In summary, this study provides *in vitro* evidence that rats pretreated with fluphenazine for 10 days, but not acutely, developed moderate but significant striatal autoreceptor supersensitivity as measured by the ability of *S*(+)-NPA, a selective DA autoreceptor agonist and very weak postsynaptic agonist, to inhibit tyrosine hydroxylase activity. In contrast, autoreceptor supersensitivity was not found with the nonselective auto- and postsynaptic receptor agonist *R*(−)-NPA. Presumably, this effect represents some modification of a presynaptic regulatory mechanism controlling DA synthesis which can occur despite a reportedly high striatal DA autoreceptor reserve in rat striatum [2, 7]. Such a mechanism, by tending to reduce synaptic availability of DA, may contribute to tolerance to the transient, early DA-synthesis stimulating actions of acutely administered neuroleptics [4], and help to counterbalance increases in postsynaptic DA receptor abundance and sensitivity associated with long-term neuroleptic treatment.

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REFERENCES

1. Wolf ME and Roth RH, Dopamine autoreceptors. In: *Dopamine Receptors* (Eds. Creese I and Fraser CM), pp. 45–96. Alan R. Liss, New York, 1987.
2. Booth RG, Baldessarini RJ, Kula NS, Gao Y, Zong R and Neumeyer JL, Presynaptic inhibition of dopamine synthesis in rat striatal tissue by enantiomeric mono- and dihydroxyaporphines. *Mol Pharmacol* **38**: 92–101, 1990.
3. Booth RG and Baldessarini RJ, Adenosine A_2 stimulation of tyrosine hydroxylase in rat striatal minces is reversed by dopamine D_2 autoreceptor activation. *Eur J Pharmacol* **185**: 217–221, 1990.
4. Baldessarini RJ, Marsh ER, Kula NS, Zong R, Gao Y and Neumeyer JL, Effects of isomers of hydroxyaporphines on dopamine metabolism in rat brain regions. *Biochem Pharmacol* **40**: 417–423, 1990.
5. Walters JR and Roth RH, Dopaminergic neurons: An *in vivo* system for measuring drug interactions with presynaptic receptors. *Naunyn Schmiedebergs Arch Pharmacol* **296**: 5–14, 1976.
6. Goldman ME and Kebabian JW, Aporphine enantiomers: Interactions with D_1 and D_2 receptors. *Mol Pharmacol* **25**: 18–23, 1984.
7. Meller E, Bohmaker K, Namba Y, Friedhoff AJ and Goldstein M, Relationship between receptor occupancy and response at striatal dopamine autoreceptors. *Mol Pharmacol* **31**: 592–598, 1987.
8. Tarsy D and Baldessarini RJ, Behavioral supersensitivity to apomorphine following chronic treatment with drugs which interfere with the synaptic function of catecholamines. *Neuropharmacology* **13**: 927–940, 1974.
9. Burt DR, Creese I and Snyder SH, Antischizophrenic drugs: Chronic treatment elevates dopamine receptor binding in brain. *Science* **196**: 326–327, 1976.
10. Cohen BM and Lipinski JF, *In vivo* potencies of antipsychotic drugs in blocking alpha 1 noradrenergic and dopamine D2 receptors: Implications for drug mechanisms of action. *Life Sci* **39**: 2571–2580, 1986.
11. Wheeler SC and Roth RH, Tolerance to fluphenazine and supersensitivity to apomorphine in central dopaminergic systems after chronic fluphenazine decanoate treatment. *Naunyn Schmiedebergs Arch Pharmacol* **312**: 151–159, 1980.

12. Bannon MJ, Bunney EB, Zigun JR, Skirboll LR and Roth RH, Presynaptic dopamine receptors: Insensitivity to kainic acid and the development of supersensitivity following chronic haloperidol. *Naunyn Schmiedebergs Arch Pharmacol* **312**: 161–165, 1980.
13. Cubeddu LX, Hoffmann IS, James MK and Niedzwiecki DM, Changes in the sensitivity to apomorphine of dopamine receptors modulating dopamine and acetylcholine release after chronic treatment with bromocriptine or haloperidol. *J Pharmacol Exp Ther* **226**: 680–685, 1983.
14. Cohen BM, Baldessarini RJ, Campbell A, Tsuneizumi T and Babb S, Persistence of antipsychotic drug effects and tissue levels. *Schizophr Res* **2**: 198, 1989.
15. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ and Klenk DC, Measurement of protein using bicinchoninic acid. *Anal Biochem* **150**: 76–85, 1985.
16. Teicher MH, Med-65 ALLFIT (Applesoft). Biomedical Computing Technology Information Center, Vanderbilt University, Nashville, TN, 1983.
17. Hyttel J and Moller-Nielsen I, Changes in catecholamine concentrations and synthesis rate in mouse brain during the supersensitivity phase after treatment with neuroleptic drugs. *J Neurochem* **27**: 314–315, 1976.

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Ketamine enantiomers and acetylcholinesterase

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Ketamine hydrochloride (2-(*o*-chlorophenyl)-2-methylaminocyclohexanone hydrochloride) is a drug with sedative, analgesic and anaesthetic properties [1]. As used clinically, ketamine is a racemic mixture, of equal concentrations of dextrorotatory (+)- and levorotatory (−)-isomers. With the aim of developing drugs with higher potency and fewer side effects, there is currently a great interest in studying the action of isolated isomers of pharmacologically active substances with two (or more) isomers. Studies on the enantiomers of ketamine in animals [2–4] and in humans, both healthy volunteers [5, 6] and patients [7], reveal a consistent picture: the (+)-isomer is more potent (approximately three times), has a more favourable therapeutic index and gives rise to less psychic emergence reactions in the postanaesthetic period than the (−)-form.

Ketamine has many effects, which have been studied at the cellular and molecular levels. The most prominent action is to antagonize, in a non-competitive way, *N*-methyl-D-aspartate receptor responses [8, 9]. Some of the molecular actions have been studied with isolated enantiomers and potency differences have been found, but in no case exceeding a factor of five, i.e. in the same range as found *in vivo*. In almost all cases, e.g. displacement of phencyclidine-binding to brain membranes [10], the (+)-isomer was found to be the most potent isomer. An exception is high affinity transport of serotonin, which was somewhat more inhibited by the (−)-isomer than by the (+)-form [11].

Our interest in ketamine is coupled to the action of anticholinesterase compounds, primarily nerve agents. One could, in the military context, expect the occurrence of "mixed casualties", i.e. patients intoxicated by nerve agent but who also have conventional injuries, prompting the use of an anaesthetic. The anaesthetic of choice must not aggravate the intoxication or counteract the action of medical treatment against the intoxication (atropine and an oxime). In an initial study on pigs, we noticed that ketamine-anaesthesized animals tolerated nerve agent better than did pentobarbital-anaesthesized animals [12], an effect most probably due to the ionic channel blocking mentioned above. We also found that ketamine was beneficial in intoxicated and antidote (atropine and HI-6)-treated guinea-pigs [13]. At the molecular level, we investigated ketamine effects on purified acetylcholinesterase (EC 3.1.1.7). Inhibition constants were determined, both for solubilized and, as ketamine has direct membrane-perturbing properties, on enzyme reconstituted into liposomes [14]. We showed that ketamine protects the enzyme from irreversible inhibition by an organophosphorus inhibitor, sarin (isopropyl methylphosphonofluoridate), and interfered with reactions of importance for the therapeutic counter-measures against organophosphorus intoxication, i.e. dealkylation ("aging") and reactivation of sarin-inhibited acetylcholinesterase [15]. In the present study we repeated these experiments, with ketamine enantiomers.

Materials and Methods

Ketamine HCl racemate and (+)-ketamine HCl were gifts from Warner Lambert Scandinavia AB/Parke-Davis, while (−)-ketamine HCl was kindly donated by Dr A. Maurset, University of Oslo, Norway. Dr J. Clement, DRES, Canada, kindly gave us HI-6 dichloride. Sarin was synthesized in the chemistry division of this institute. Acetylcholinesterase was purified from bovine brain [14] and had a specific activity of 30 μkat/mg. The inhibition constants for the two isomers against acetylcholinesterase were determined as described recently [14] and the reaction constants for sarin inhibition, reactivation and aging according to [15].

Results and Discussion

The two enantiomers of ketamine are both inhibitory towards acetylcholinesterase and the inhibition is of a mixed type (Table 1) thus affecting both the binding of the substrate and the velocity for its hydrolysis. We found that the (−)-form was slightly more potent, having a lower K_i,