Analogs of Parasympathetic Neuroeffectors. III. Synthesis and Study of Cholineselenol and Related Compounds[1,2]

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The depolarizing activity of acetylselenocholine is exceeded by that of its enzymatic hydrolysis product, cholineselenol. The synthesis of cholineselenol and homocholineselenol, using a hypophosphorous acid reduction of the corresponding diselenides, is reported.

The nature of the receptor sites in acetylcholinesterase and in the cholinergic receptor and the changes that occur when an active substance attaches itself to these macromolecules have been the subject of intensive investigation for a number of years. The recent synthesis of acetylselenocholine[3] prompted a comparative pharmacological study of acetylcholine, acetylthiocholine, and acetylselenocholine. Isologous esters of the above type have rather similar sizes, so that the ability to *fit* large receptor molecules should remain largely unaltered, while their electron distribution may be rather different,[4,5] thus affecting the ability to bind to receptor sites or to induce configurational changes in the receptor proteins. In the guinea pig ileum and the frog rectus abdominis preparations it was found[6] that all three compounds exerted cholinergic effects. Addition of cholinesterase inhibitors greatly enhanced the response to acetylcholine, while the responses to acetylthio- and acetylselenocholine were reduced. This effect was shown to be due to high activities of the enzymatic hydrolysis products, cholinethiol and cholineselenol, while choline was essentially inert. Isolation of pure cholineselenol from enzymatic or alkaline hydrolysis reactions proved most difficult, due to the extreme ease of oxidation by atmospheric oxygen to the corresponding diselenide. An independent synthesis was, thus, desirable.

Only very few selenols have ever been isolated in a state of high purity without contamination with diselenides. Aliphatic selenols are generally prepared by the reduction of diselenides, the latter being comparatively stable compounds which may be obtained by a variety of procedures. Reductive cleavage of the selenium–selenium bond has been accomplished by the use of sodium in liquid ammonia,[7] or, more recently, by the use of sodium borohydride[3,8] or lithium aluminum hydride.[9,10] Reactions of selenium compounds with alkali metals can result in undesirable cleavages of carbon–selenium bonds.[11]

Application of the above methods to the reduction of choline diselenide failed, since the reduction products could not be separated conveniently from the reagents used. Since the oxidation of selenols is less rapid in acid than in base, a search for suitable acidic reducing agents was instituted. The latter should not cause decomposition of onium compounds and permit their isolation in as few steps as possible.

Such a reagent has now been found in the form of hypophosphorous acid.[2,12] Treatment of a suspension of bis(2-trimethylammoniumethyl) diselenide diiodide (the diselenide form of selenocholine) in methanol with slightly more than 1 molar equiv. of hypophosphorous acid at the boiling point of the solvent led to a rapid decoloration of the initially yellow solution. On cooling, the colorless cholineselenol iodide was obtained in crystalline form.

Cholineselenol iodide liberated CO_2 from bicarbonate solution and consumed 1 equiv. of iodine in acidic solution and 4 equiv. in the presence of sodium bicarbonate, of which three were liberated again on acidification. The stoichiometry indicated a selenol which is oxidized to a diselenide in the first oxidation step, to a seleninic acid in the second step, which in turn is reduced by hydrogen iodide to the diselenide according to eq. 1–3.[13]

$$2RSeH + I_2 \longrightarrow (RSe)_2 + 2HI \quad (1)$$

$$(RSe)_2 + 3I_2 + 8NaHCO_3 \longrightarrow 2RSeO_2Na + 6NaI + 8CO_2 + 4H_2O \quad (2)$$

$$2RSeO_2H + 6HI \longrightarrow (RSe)_2 + 3I_2 + 4H_2O \quad (3)$$

Cholineselenol reduced dichlorophenol indophenol. Bright yellow silver and lead salts, contaminated by silver and lead iodide, could be precipitated from aqueous solution. Air oxidation of the selenol yielded choline diselenide diiodide, and acylation with benzoyl chloride or acetic anhydride in the presence of bicarbonate buffer yielded acetylselenocholine[3] and benzoylselenocholine[3] with characteristic ultraviolet absorption peaks at 249 and 285 mμ, respectively.

The high acidity of hydrogen selenide[14] (pK_a = 3.73), compared to that of hydrogen sulfide[14] (pK_a = 6.94), suggested that selenols should be considerably more acidic than isologous thiols. Since biological

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activity and dissociation constants of biologically effective compounds are frequently related, it seemed of interest to determine the relative acid strengths of cholinethiol and cholineselenol. While the former compound has a pK_a of 7.7,[15] that of its selenium isolog was found to be 4.68 ± 0.05. The fact that cholineselenol at physiological pH exists predominantly in the selenomercaptide form presumably accounts for the difficulties encountered in keeping this compound reduced under such conditions.

During a recent study[16] of the depolarizing activities of oxo, thio, and seleno compounds related to acetylcholine and choline in the electroplax preparation, it was noted, that while the depolarizing activities of acetylcholine and choline differed widely, those of homocholine and acetylhomocholine were rather similar.[17] This finding suggested the investigation of the thio and seleno analogs of acetylhomocholine, homocholine, and methoxyhomocholine.

Bis(3-dimethylaminopropyl) diselenide was synthesized by the reaction of 3-dimethylaminopropyl chloride hydrochloride with potassium selenosulfate in aqueous solution, followed by hydrolysis of the intermediate seleno–Bunte salt. Reduction of the diselenide with sodium borohydride, followed by treatment with benzoyl chloride in sodium bicarbonate buffer or acetic anhydride in methanol solution yielded 3-dimethylaminopropyl selenolbenzoate and 3-dimethylaminopropyl selenolacetate, respectively, which were used without further purification in the next step. Treatment of the tertiary amines with methyl bromide or methyl iodide in ether solution yielded 3-trimethylammoniumpropyl selenolbenzoate bromide, 3-trimethylammoniumpropyl selenolacetate bromide, and bis(3-trimethylammoniumpropyl) diselenide diiodide. The reduction of the latter compound with hypophosphorous acid was carried out as described for the preparation of cholineselenol, giving 3-trimethylammoniumpropylselenol iodide in nearly quantitative yield.

Unfortunately, the high oxidizability of cholineselenol interfered with studies of the depolarizing activity of this compound in the improved single-cell electroplax preparation.[18] However, a study in this system of acetylselenocholine in the presence and absence of eserine, an acetylcholinesterase inhibitor, indicated[16] that, as in the guinea pig ileum and frog rectus abdominis preparations,[6] the depolarizing activity of cholineselenol exceeds that of its acetyl ester.

Experimental Section

2-Trimethylammoniumethylselenol Iodide (Cholineselenol Iodide).—A suspension of bis(2-trimethylammoniumethyl) diselenide diiodide[3] (5.9 g., 0.01 mole) in methanol (30 ml.) and hypophosphorous acid (2 ml. of 50% aqueous solution, 0.015 mole) was warmed to 50° in a centrifuge tube until a clear, nearly colorless solution was obtained. The tube was then cooled in ice for 30 min. and the colorless crystals were collected by centrifugation. The product was immediately recrystallized from 20–25 ml. of methanol to which 2 drops of hypophosphorous acid had been added, washed with absolute ethanol and peroxide-free ether in the centrifuge tube, and dried under vacuum. Cholineselenol iodide (3.2 g., 54%) was obtained as colorless needles, m.p. 207° dec.[19]

Anal.[20] Calcd. for $C_5H_{14}INSe$: C, 20.42; H, 4.80; I, 43.16; N, 4.76; Se, 26.85. Found: C, 20.24; H, 4.66; I, 43.80; N, 5.04; Se, 26.87.

The acid dissociation constant of cholineselenol (pK_a = 4.68 ± 0.05) was determined as was that of cholinethiol (pK_a = 7.77, lit.[15] 7.7) by potentiometric titration in an atmosphere of pure nitrogen using a Radiometer Autotitrator (The Radiometer Co., Copenhagen, Denmark).

Bis(3-dimethylaminopropyl) Diselenide Dihydrochloride.—A solution of 3-dimethylaminopropyl chloride hydrochloride (54 g., 0.35 mole) in water (100 ml.) was added dropwise, with stirring, to a refluxing solution of potassium selenosulfate (from 27 g. of finely powdered selenium and 70 g. of potassium sulfite). The pale yellow solution was heated for about 10 more min. and then concentrated HCl (100 ml., 1.2 moles) was dropped in slowly with continued stirring. The bright yellow reaction mixture was evaporated to a small volume under reduced pressure, ethyl alcohol (200 ml.) was added, and most inorganic salts were removed by filtration. The yellow filtrate was again evaporated to dryness, the residue was taken up in water and made strongly alkaline with 3.5 *N* NaOH solution. The oily precipitate was extracted with ether, the ether solution was washed several times with water, the amine was re-extracted with 3 *N* HCl, and the final aqueous solution was evaporated to dryness under reduced pressure. The yellow residue was recrystallized from a mixture of methanol and acetone to yield bis(3-dimethylaminopropyl) diselenide dihydrochloride (45.3 g., 65%), m.p. 204–205.5°.

Anal. Calcd. for $C_{10}H_{26}Cl_2N_2Se_2$: C, 29.79; H, 6.50; N, 6.95; Se, 39.17. Found: C, 29.51; H, 6.20; N, 6.86; Se, 39.50.

3-Acetylselenopropyltrimethylammonium Bromide (Acetylselenohomocholine Bromide).—A suspension of bis(3-dimethylaminopropyl) diselenide dihydrochloride (2.0 g., 0.005 mole) in methanol (3 ml.) was treated with solid $NaBH_4$ until a colorless solution was obtained. Acetic anhydride (10 ml.) was then added, and the solution was evaporated to dryness after the vigorous reaction had subsided. The oily residue was triturated with anhydrous ether and the filtered ethereal solution was mixed with methyl bromide (1 g., 0.011 mole). The reaction mixture was kept overnight at room temperature and the colorless crystals (2.2 g., 73%) were recrystallized from acetone; m.p. 135–137°.

Anal. Calcd. for $C_8H_{18}BrNOSe$: C, 31.69; H, 5.99; N, 4.62; Se, 26.05. Found: C, 31.51; H, 5.86; N, 4.84; Se, 25.70.

3-Benzoylselenopropyltrimethylammonium Bromide.—An ice-cold solution of 3-dimethylaminopropylselenol [from 4.0 g. (0.01 mole) of bis(3-dimethylaminopropyl) diselenide dihydrochloride by reduction with $NaBH_4$] in water (30 ml.) was stirred with $NaHCO_3$ (10 g.) and benzoyl chloride (5 ml.) until the smell of benzoyl chloride became faint. The mixture was brought to pH 9.5 by addition of NaOH and quickly extracted with peroxide-free ether. The ethereal solution was washed with saturated aqueous Na_2SO_3, dried (Na_2SO_4), and mixed with methyl bromide (5 ml.). The colorless precipitate (5.3 g., 76%) was collected after 17 hr. at room temperature. Recrystallized from absolute ethanol, the colorless needles had m.p. 218–219°.

Anal. Calcd. for $C_{13}H_{20}BrNOSe$: C, 42.75; H, 5.52; N, 3.84; Se, 21.62. Found: C, 42.60; H, 5.44; N, 3.98; Se, 21.86.

3-Methylselenopropyltrimethylammonium Iodide (Methylselenohomocholine Iodide).—Bis(3-dimethylaminopropyl) diselenide dihydrochloride (2.03 g., 0.005 mole) was suspended in methanol (30 ml.) and reduced to the corresponding selenol by addition of solid $NaBH_4$ (approximately 0.8 g., 0.02 mole) until the mixture appeared colorless. Acetone (100 ml.) was then added, followed after a few seconds by methyl iodide (4.2 g., 50% excess). The solution was filtered as quickly as possible from precipitated salts and allowed to remain at room temperature for 30 min. An equal volume of ether was then added and the crystalline product was collected by filtration. The methylseleno compound (3.1 g., 92%) melted at 227–230° dec. after recrystallization from ethanol and ether.

Anal. Calcd. for $C_7H_{18}INSe$: C, 26.10; H, 5.63; N, 4.34. Found: C, 26.06; H, 5.56; N, 4.14.

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Bis(3-trimethylammoniumpropyl) Diselenide Dilodide (Homocholine Diselenide Diiodide).—A solution of bis(3-dimethylaminopropyl) diselenide [from 10.0 g. (0.025 mole) of the dihydrochloride by extraction from alkaline solution] in dry ether (100 ml.) was mixed with methyl iodide (5 ml.). The reaction was allowed to proceed overnight at room temperature and the yellow precipitate (15.4 g., 100%) was recrystallized from methanol, m.p. 211–213° dec.

Anal. Calcd. for $C_{12}H_{30}I_2N_2Se_2$: C, 23.47; H, 4.92; N, 4.56; Se, 25.72. Found: C, 23.73; H, 4.75; N, 4.75; Se, 26.01.

3-Trimethylammoniumpropylselenol Iodide (homocholineselenol iodide) was obtained by a reduction procedure similar to the one described for cholineselenol iodide. The homocholine diselenide diiodide (3.0 g., 0.005 mole) in absolute ethanol (25 ml), reduced with hypophosphorous acid (2 ml.) at the boiling point of the solvent, yielded colorless needles of the desired selenol iodide (2.8 g., 93%), m.p. 169–170°.

Anal. Calcd. for $C_6H_{16}INSe$: C, 23.39; H, 5.23; N, 4.55; Se, 25.63. Found: C, 23.48; H, 5.23; N, 4.79; Se, 25.26.

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Structure–Activity Relationship of Some New Analogs of Pethidine

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The synthesis and analgesic activity are described for 32 N-substituted analogs of pethidine, which may be grouped in seven series with the structure 1-R$(CH_2)_n$-4-C_6H_5-piperidine-4-$CO_2C_2H_5$: series A (n = 4–7, R = Me); B (n = 1–5, R = CH_2OH); C (n = 1–5, R = CH_2OEt); D [n = 1, R = $CH_2O(CH_2)_{0,2-5}CH_3$]; E (n = 1–3, R = CH_2OCH_2-2-furyl); F (n = 0–4, R = CH_2-2-furyl); G (n = 0–4, R = cyclopentyl). A comparison of the structure–activity relationship within and between these series is made. Analgesic activities range from less than one-tenth to twenty-eight times that of pethidine and increase regularly with increasing chain length of the N-substituent to a maximum, after which activity falls off at a similar rate. Maximum analgesic activity is found when the N-substituent skeleton consists of six or seven atoms, with a chain length of 7–9 Å. Possible explanations for these findings are discussed.

Many N-substituted derivatives of norpethidine (ethyl 4-phenyl-4-piperidinecarboxylate) have been synthesized in recent years,[2–7] and several of these substances were found to be more potent than the parent substance pethidine. Thus morpheridine, first synthesized in these laboratories in 1953,[4] was twice as potent (on a weight basis) as pethidine.[8] Subsequently, derivatives in which the N-substituent contained an open chain[9] or a cyclic ether linkage[10] were described. Systematic modifications in the chain length of the substituent attached to the nitrogen atom have now been made in seven series of derivatives (see Table I). The effect of these modifications upon analgesic potency within each series indicates that a definite relationship exists between chain length of the N-substituent and analgesic potency.

Chemistry.—The compounds of series C–F have been described previously[9,10]; the other substances were prepared by alkylation of norpethidine with the appropriate halide. Some n-alkylnorpethidines (series A) were described[11] while this work was in progress. The ω-chloroalkanols required for the preparation of the compounds of series B were synthesized from the respective glycols or from tetrahydrofuran (*cf.* Experimental Section). The cyclopentylalkyl bromides (for series G) were all made from cyclopentyl bromide, the chain being lengthened by the action of formaldehyde or of ethylene oxide on the appropriate Grignard reagent.

Pharmacology.—Analgesic activity was measured in weanling male rats of the Wister strain weighing 50–60 g. by a method[12] based on that of Green and Young.[13] This method uses pressure on the tip of the tail as the pain stimulus, and this is the major difference between the method we have adopted and the hot-plate method used by Janssen and Eddy.[14] Five groups of 8 or 10 rats were used for each evaluation, and each animal was used as its own control. The mean pressure required to produce a pain response before and after drug administration was determined; if the postdrug threshold pressure were equal to or greater than twice the predrug threshold, the drug was judged to be producing an analgesic effect. The ED_{50} and 95% confidence limit for analgesia were determined using the standard probit analysis. All compounds were injected subcutaneously and the analgesic activity was determined 30 min. after injection. All ED_{50} values were expressed as μmoles of base/kilogram of body weight and the potency ratios were compared on an equimolar basis, with pethidine taken as unity. This method permits a direct comparison with the figures obtained by Janssen and Eddy.

The analgesic activities of the seven series of norpethidine derivatives investigated are shown in Table I. Figures 1 and 2 show plots of molar potency (relative to pethidine taken as unity) on a log scale against chain length of N-substituent. The number of side-chain

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