The consumption of NADPH by the microsomes was increased by the addition of aminopyrine only in the case of phenobarbital-treated rats. Presumably there are several reactions in which NADPH may be consumed, and the increase in rate when aminopyrine is added simply reflects the fact that phenobarbital treatment has so increased the capacity of the microsomes to activate NADPH that there is not enough endogenous substrate present to allow the reaction to proceed at maximum rate. The protein isolated per mg liver was significantly increased only in the case of phenobarbital treatment.

None of the four dogs treated with ethchlorvynol showed a significant shortening of the half-life of bishydroxycoumarin (Table 2). In agreement with other reports,[1] when three of these dogs were later treated with phenobarbital, two showed a highly significant decrease in bishydroxycoumarin half-life. It should be noted that dog 2, which showed no response to phenobarbital, had a control half-life of bishydroxycoumarin half as long as any of the other dogs. Perhaps prior to the experiment he had accidentally been exposed to an insecticide or another inducer. These agents have been shown to exert extremely long-lasting effects in dogs.[13]

These data suggest that ethchlorvynol neither induces nor inhibits the liver enzymes that metabolize drugs. Further studies in man at clinical doses will be necessary to confirm this point.

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Sulphur and selenium compounds related to acetylcholine and choline—VIII. Comparative studies of succinoylcholine, succinoylthiolcholine and succinoylselenolcholine*†

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IN AN attempt to obtain information about the structure of the active sites of acetylcholinesterase and of the depolarizing membrane of conducting tissue, the synthesis and study of sulfur and selenium isologs of acetylcholine, choline and of related compounds have been undertaken.[1–7] Since replacement

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† For earlier papers in this series, see References 4-10.

of oxygen by sulfur or selenium alters molecular size relatively little, but brings about major changes in electron density amenable to study by physiochemical methods,[8, 9] one is dealing here with a series of isosteric, electronically defined compounds. Replacement of oxygen by sulfur or selenium greatly modified the biological activities of choline and homocholine or their esters.[2, 4, 7]

Since succinoylcholine[10] has found widespread clinical use[11] as a neuromuscular blocking agent, the effects of replacing the side-chain oxygens of this compound with sulfur and selenium appeared to be worthy of study. The synthesis of succinoylthiolcholine has been reported by a Czechoslovakian[12] and by an Armenian[13] laboratory; however, few details were provided except for the statement[12] that succinoylthiolcholine possessed no practical curare-like activity. Succinoylselenolcholine had not been synthesized previously.

MATERIALS AND METHODS

Succinoylthiolcholine dihydroiodide. A solution of 8 g (0·05 mole) of succinoyl chloride in 100 ml of methylene chloride was added dropwise, with vigorous stirring, to a solution of 10 g (0·095 mole) of 2-dimethylaminoethylthiol in 100 ml of methylene chloride. The white precipitate was removed by filtration, washed with ether, and dissolved in a minimal amount of cold water. The solution was treated with 40 ml of aqueous saturated sodium carbonate and extracted four times with 25-ml portions of ethyl acetate. The organic layers were combined, washed with saturated sodium sulfate solution, and dried over anhydrous sodium sulfate. After filtration, the filtrate was treated with 6 ml (0·05 mole) of methyl iodide. After standing at room temperature for 1 day, the solution yielded 23 g (80%) of pink, waxy crystals. After being recrystallized three times from water, the product melted at 216°.

Anal. Calculated: $C_{14}H_{30}N_2O_2S_2I_2$: C, 29·17; H, 5·30; S, 11·13; N, 4·86; I, 44·04. Found: C, 29·00; H, 5·42; S, 10·83; N, 4·62; I, 44·27.

Succinoylselenolcholine dihydroiodide. A solution of 10 g (0·028 mole) of *bis*(2-dimethylaminoethyl)diselenide in 130 ml of water was treated under a nitrogen atmosphere with 2 g (0·05 mole) of sodium borohydride. To the resulting colorless solution was added 4·4 g (0·051 mole) of sodium bicarbonate, followed by 4·1 g of succinoyl chloride. The latter reagent was added dropwise with constant stirring. After completion of the reaction, 35 ml of aqueous saturated sodium carbonate was added, followed by extraction with four 20-ml portions of ethyl acetate. Quaternization and purification procedures were analogous to those described in the above synthesis of succinoylthiolcholine. A crude yield of 10·5 g (70%) of small yellowish-white crystals was obtained; these were recrystallized four times from a 25:1 water:acetone solution. The product melted at 185–186°.

Anal. Calculated: $C_{14}H_{30}N_2O_2Se_2I_2$: C, 25·09; H, 4·51; N, 4·18; Se, 23·57. Found: C, 24·91; H, 4·84; N, 4·13; Se 23·48.

Freshly synthesized succinoylcholine dihydroiodide was used in all experiments.

Phrenic nerve stimulated rat diaphragm preparation. The preparation described by Bülbring[14] was used. The muscle was mounted in a 20-ml bath containing McEwen's solution[15] maintained at 37 ± 0·2° and aerated with 95% oxygen and 5% carbon dioxide. The phrenic nerve was stimulated by supramaximal rectangular pulses of 0·3 msec applied at a frequency of 6/min. Muscle twitches were recorded by a spring-loaded, semi-isometric, side-writing lever writing on a smoked drum.

RESULTS

The effects of succinoylthiolcholine and succinoylselenolcholine as neuromuscular blocking agents were studied in relation to the effects induced by succinoylcholine in the phrenic nerve stimulated rat diaphragm preparation. Since succinoylcholine is relatively stable and its effects can be reversed readily by washing the preparation, this compound was used as a standard to determine the sensitivity of the system and to indicate normal or abnormal behaviour of the preparation at any given time.

Typically, 0·3 ml of millimolar succinoylcholine introduced into the 20-ml bath induced an immediate slight blocking action that increased to a maximum of 20–30 per cent blockade within 6 min. This effect was reversed rapidly by washing.

Succinoylthiolcholine and succinoylselenolcholine required a longer contact time (9–12 min) to induce maximal blockade than did succinoylcholine. The effect of succinoylthiolcholine (25 per cent block due to 0·2 ml of 0·01 M solution) was more persistent than the effect of the oxygen analog; however, after 40 min of periodic washing the effects of succinoylthiolcholine disappeared completely.

Succinoylselenolcholine (0·3 ml of millimolar solution) also induced blockade (20–25 per cent). The effect of the selenolester proved to be irreversible and was not affected by periodic washing for periods up to 1 hr.

When succinoylthiolcholine or succinoylselenolcholine was introduced into an organ bath containing McEwen's solution and prostigmine ($7{\cdot}5 \times 10^{-7}$M), the blocking potencies of these compounds were enhanced. Since prostigmine is an esterase inhibitor, this observation suggests that the sulfur and selenium compounds, like succinoylcholine, are acting as depolarizing neuromuscular blocking agents.

DISCUSSION

The irreversible effect of the depolarizing neuromuscular blocking action of succinoylselenolcholine, in contrast to the reversibility of the effects of its sulfur and oxygen isologs, is worthy of note. Similarly, it has been observed recently[16] that in the giant axon of the squid pretreated with cottonmouth moccasin venom, 2-dimethylaminoethylselenolbenzoate but not the corresponding thiolbenzoate or benzoate, induced irreversible blockade of electrical activity.

It has been shown that selenolesters react considerably more rapidly with nucleophiles such as amines and mercaptans, or phosphate than do thiolesters or esters.[5, 9] Selenolesters have also been found capable of the reductive acylation of disulfides.[17, 18] It thus seems plausible that the irreversible action of selenolesters on some conducting tissues might be due to the acylation of nucleophilic groups belonging to the active site of the target receptor protein. Further experiments with labeled compounds will be required to prove or disprove this point.

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