The inhibitory effect of glycopyrrolate on human plasma-cholinesterase

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The in vitro inhibitory effect of glycopyrrolate and atropine on the hydrolysis of benzoylcholine at 50.0 μ M concentration was determined in the plasma of 6 healthy volunteers at 37° C and at 240 nm by the ultraviolet spectrophotometric method of Kalow. The 50 per cent inhibitory concentration (I_{50}) of glycopyrrolate was found to be 1.0 mM and the I_{50} of atropine was 0.9 mM. This inhibitory effect of glycopyrrolate and atropine is moderate but its interaction with the in vivo hydrolysis of succinylcholine or that of ester-type of local anaesthetics in patients should be further studied. Although both glycopyrrolate and atropine inhibit PChE to the same extent, glycopyrrolate should be utilized in patients in whom inhibition of brain PChE or central effects ought to be avoided, since glycopyrrolate does not penetrate the blood-brain barrier.

Key words

PARASYMPATHETIC NERVOUS SYSTEM: atropine, glycopyrrolate; ENZYMES: plasmacholinesterase, inhibition, hydrolysis; NEUROMUSCULAR RELAXANTS: succinylcholine.

It is well known that succinylcholine and ester-type of local anaesthetics, e.g., procaine and chlorprocaine are hydrolyzed by human plasma cholinesterase (PChE, E.C. 3.1.1.8) and their enzymatic hydrolysis is responsible for the termination of their toxicity.^{1,2} Drugs that inhibit PChE or genetically abnormal dibucaine-resistant PChE may, therefore, increase the toxicity of succinylcholine and local anaesthetics.³⁻⁵ Consequently, anticholinergics, like atropine and glycopyrrolate are frequently used intravenously to prevent the bradycardia and cardiac arrhythmias caused by IV succinylcholine. Previously, atropine was reported to inhibit PChE activity in a concentration of 1.0 mmol/L to 50 per cent by Kaswin.⁶

The recent utilization of physostigmine in clinical anaesthesia for the reversal of the central effects of diazepam focused our attention on the possible interaction of anticholinergics with anaesthetic agents, analgesics and tranquilizers in the central nervous system. Some cholinomimetic agents even exert antinociceptive effects that are abolished by atropine.⁷ Reactive behaviour can also be altered by cholinomimetics⁸ and atropine can reverse this central effect, but quaternary derivatives of atropine such as methylatropine remain ineffective, since they cannot penetrate the blood-brain barrier. Narcotic antagonists are also capable of reversing the antinociceptive effects of cholinomimetics.^{7,9} The routine use of glycopyrrolate in place of atropine prevents this interference with the central effects of physostigmine and other drugs frequently used following anaesthesia,¹⁰ while exerting its protecting effect from peripheral vagal stimulation associated with physostigmine. Although one should not expect any effect of glycopyrrolate on brain cholinesterases, since it does not get to the brain, its effect on plasma PChE should be known

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FIGURE 1 Inhibitory effect of glycopyrrolate on human plasma cholinesterase. $I_{50} = 1.0$ mM with benzoylcholine substrate.

FIGURE 2 Inhibitory effect of atropine on human plasma cholinesterase. $I_{50} = 0.9$ mM with benzoylcholine substrate.

but has not been previously investigated. Therefore this study was undertaken.

Methods

The source of plasmacholinesterase was freshly obtained heparinized plasma of six healthy volunteers with genetically normal PChE, proven by dibucaine number determinations. Commercially available benzoylcholine hydrochloride was used in a concentration of $50.0 \mu\text{M}$. Glycopyrrolate methobromide or atropine sulfate in concentrations ranging from $1.0 \mu\text{M}$ to 10.0 mM, were incubated with the plasma for 20 minutes at 37°C before the addition of the substrate. The enzymatic hydrolysis rate was determined by the method of Kalow¹¹ in an ultraviolet spectrophotometer in a phosphate buffer of $\text{pH} = 7.4 \pm 0.02$ at 240 nm and at $37 \pm 0.05^\circ\text{C}$. The means of duplicate determinations were calculated and presented.

Results

As Figure 1 indicates, the 50 per cent inhibitory concentration (I_{50}) of glycopyrrolate was found to be 1.0 mM when the $-\log |M|$ concentrations of glycopyrrolate were plotted against the per cent inhibition of benzoylcholine hydrolysis. Figure 2 shows that the I_{50} of atropine was 0.9 mM; that is almost identical to that of glycopyrrolate.

Discussion

Glycopyrrolate and atropine possess low inhibitory effect on human PChE, therefore it is unlikely that

PChE *in vivo* would be inhibited to an extent that interferes with the hydrolysis of succinylcholine and/or local anaesthetics of the ester-type. Nonetheless, this possible interaction of glycopyrrolate and atropine with muscle relaxants and local anaesthetics should be further studied in patients. Since glycopyrrolate does not cross the blood-brain barrier, it should be void of central anticholinesterase effect such as the inhibition of brain PChE at the receptor sites. Such an interaction of atropine on the CNS cannot be excluded, since it freely passes the blood-brain barrier and, therefore, may inhibit brain PChE, in addition to its other central pharmacologic effects. This lack of central effect of glycopyrrolate makes it a choice of anticholinergic drugs in anaesthetic practice in patients in whom this interaction should be avoided.

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Résumé

L'effet inhibiteur in vitro du glycopyrrolate a été déterminé sur l'hydrolyse de la benzoylcholine à 50.0 µM dans les plasmas de seize volontaires sains à 37° C et à 240 nm par la méthode spectrophotométrique à ultraviolet de Kalow. La concentration inhibitrice de 50 pour cent (I₅₀) du glycopyrrolate sur la cholinestérase plasmatique a été trouvée égale à 1.0 mM. La concentration inhibitrice de 50 pour cent (I₅₀) de l'atropine a été trouvée égale à 0.9 mM. L'effet inhibiteur du glycopyrrolate et de l'atropine est modéré, mais l'interaction potentielle avec l'hydrolyse in vivo de la succinylcholine ou des analgésiques locaux du type-ester doit être étudiée chez les patients anesthésiés. Bien que le glycopyrrolate et l'atropine inhibent également la cholinestérase plasmatique, le glycopyrrolate doit être utilisé chez les patients dont l'inhibition de la cholinestérase plasmatique du cerveau ne doit pas être permise, puisque le glycopyrrolate ne peut pas pénétrer la barrière hémato-encéphalique.