SHORT COMMUNICATIONS

IN VITRO DISPLACEMENT OF VASOACTIVE MEDIATORS FROM PLASMA PROTEINS: A POSSIBLE MECHANISM FOR PSEUDO-ALLERGIC REACTIONS TO NEUROMUSCULAR BLOCKING DRUGS

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SUMMARY

We have studied the release of prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) and histamine from serum proteins by neuromuscular blocking drugs using equilibrium dialysis, with tracer quantities of radio-labelled mediators as probes. Small concentrations (0.05–0.25 mmol litre$^{-1}$) of competitive neuromuscular blocking drugs displaced 16–67% of bound histamine. Greater concentrations of suxamethonium (2 mmol litre$^{-1}$) were required for histamine displacement (19%). There was a significant release of PGF$_{2\alpha}$ by atracurium 1 mmol litre$^{-1}$ and pancuronium 0.69 mmol litre$^{-1}$. These findings suggest an alternative mechanism of histamine release by neuromuscular blocking drugs which may be relevant to adverse reactions during use. (Br. J. Anaesth. 1992; 69: 508-510)

KEY WORDS


Adverse reactions to neuromuscular blocking drugs are sometimes categorized as pseudo-allergic because they appear to produce their effect by mechanisms similar to those of the immediate immune response [1]. Histamine has been identified as an important instigator of adverse reactions to neuromuscular blocking drugs and its release has been attributed to the direct action of these drugs on histamine-containing cells [2]. Release of vasoactive mediators from plasma protein in response to neuromuscular blocking drugs has not been considered as an alternative mechanism. This in vitro study has investigated the effects of neuromuscular blocking drugs on prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) and histamine binding in serum.

METHODS AND RESULTS

Binding of PGF$_{2\alpha}$ and histamine was measured by equilibrium dialysis [3] in serum from healthy volunteers, who were not taking any medication. Tritiated PGF$_{2\alpha}$ 20 µl (0.74 KBq) (6.55 TBq mmol$^{-1}$; Amersham plc) was added to serum 1.5 ml diluted 1:1 in phosphate buffered saline (PBS). Serum samples in visking tubing were dialysed against an equal volume of PBS containing 0.1% sodium azide for 36 h before the unbound PGF$_{2\alpha}$ was measured in a scintillation counter. To measure histamine binding, tritiated histamine dihydrochloride 20 µl (0.74 KBq) (1.4 TBq mmol$^{-1}$; New England Nuclear) was added to undiluted serum and dialysed against PBS containing 1% phenol. Neuromuscular blocking drugs were added (in 30 µl of distilled water) to the serum samples before dialysis, although larger volumes of injectable drug preparations were also used. Injectable drug preparations consisted of alcuronium chloride (Roche), pancuronium and vecuronium bromides (Organon Teknika), atracurium besylate and tubocurarine chloride (Calmic). Atracurium besylate, suxamethonium chloride and tubocurarine chloride were also provided as pure agents by Burroughs Wellcome. Pancuronium bromide, histamine dihydrochloride and sodium salicylate were purchased from Sigma Chemical Co. Dorset, England.

Under these experimental conditions, 44.0 (SD 7.3)% (n = 20) of the added PGF$_{2\alpha}$ and 13.4 (4.1)% (n = 20) of histamine became associated with serum proteins. Atracurium and pancuronium, in final concentrations of 1 and 0.69 mmol litre$^{-1}$, respectively, inhibited PGF$_{2\alpha}$ binding by > 20%. At this concentration, atracurium had an inhibitory potency similar to that of salicylate. The binding of PGF$_{2\alpha}$ was increased significantly by alcuronium. The neuromuscular blocking drugs were more potent at displacing histamine from plasma protein than at displacing PGF$_{2\alpha}$. With the exception of suxamethonium, at 0.05 mmol litre$^{-1}$ all inhibited histamine binding by > 25%; greater drug concentrations (0.25 mmol litre$^{-1}$) were more inhibitory. In a concentration of 0.05 mmol litre$^{-1}$, the histamine releasing effects of both atracurium and tubocurarine were similar to that of "cold" histamine 0.01 mmol litre$^{-1}$. Greater concentrations of suxamethonium (2 mmol litre$^{-1}$) displaced 19% of the histamine associated with serum protein, although this effect

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was not statistically significant. Salicylate 1 mmol litre⁻¹ increased the quantity of histamine associated with serum protein. In contrast, the same concentration of cold histamine displaced nearly 90% of the radio-labelled histamine.

**COMMENT**

It is difficult to accept that immediate hypersensitivity is the sole cause of adverse reactions to neuromuscular blocking drugs, because of the small concentrations of specific antibody in reactive patients, the observed cross-reaction to different drugs and the apparent development of immunity with no previous exposure [4]. While there is apparent consensus on the important role of histamine in these adverse reactions, the mechanism of its release remains unknown. The distinction between allergic and pseudo-allergic reactions is more clear with other drugs. Aspirin sensitivity is a more typical pseudo-allergic reaction because of the absence of IgE antibodies in reactive patients. Aspirin and other non-steroidal anti-inflammatory drugs induce adverse reactions through inhibition of cyclo-oxygenase or by their protein binding characteristics, which displace PGF₂α from plasma protein [3]. The release of this bronchoconstricting prostaglandin may be relevant to the development of aspirin-induced asthma. As this displacement mechanism may be applicable to pseudo-allergic reactions in general, it was of interest to investigate the potential of neuromuscular blocking drugs for the displacement of PGF₂α and histamine from plasma protein.

We demonstrated that small concentrations of neuromuscular blocking drugs can displace radio-labelled histamine from serum protein in vitro. The inhibitory effects of these drugs on histamine binding increased with concentration. Furthermore, salicylate and cold histamine showed specificity in inhibiting the binding of radio-labelled PGF₂α and histamine, respectively. Although suxamethonium displaced histamine only at greater concentrations, the displacement mechanism is still relevant to this drug, which is used in clinical practice in concentrations greater than those used for competitive neuromuscular blocking drugs. The concentration of suxamethonium in preparations for injections is 126 mmol litre⁻¹(50 mg ml⁻¹). In contrast, the concentrations of the other tested drugs ranged from 2.7 mmol litre⁻¹ (pancuronium: 2 mg litre⁻¹) to 13.0 mmol litre⁻¹ (tubocurarine: 10 mg ml⁻¹). PGF₂α binds to plasma albumin, a protein with many low affinity binding sites for drugs and endogenous substances, including histamine. The large plasma concentration of albumin gives this protein a binding capacity that is not readily saturated. Almost all (approximately 99%) PGF₂α in plasma, of the order of 100 pg ml⁻¹, is protein-bound [3]. Knowledge about histamine binding to plasma proteins in normal subjects is limited. A peptide responsible for binding histamine dissociates from albumin during coagulation or enzymic digestion [5]. Although the basic quaternary amine structure of neuromuscular blocking drugs should promote their binding to alpha-1-acid glycoprotein (AAG), both alcuronium and gallamine are bound predominantly by plasma albumin [6]. However, increased resistance to the action of atracurium in patients may be associated with an increase in AAG concentration. The binding characteristics of albumin and AAG may form a natural protective mechanism which reduces the effects of released vasoactive mediators. The presence of these protein-bound mediators may not become evident unless they are displaced by increased drug concentrations associated with i.v. injection. Our results provide some support for this hypothesis and suggest that the displacement mechanism may be relevant in a proportion of adverse reactions to neuromuscular blocking drugs.

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REFERENCES


