Paraben Preservatives but Not Succinylcholine
Are Cerebral Vasodilators In Vitro

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Since the increase in intracranial pressure produced by succinylcholine is temporally associated with intravenous administration, we investigated *in vitro* a possible direct cerebrovascular effect of this nicotinic drug. Isometric responses were recorded from dog and guinea pig basilar artery rings suspended in modified Krebs' solution at 37° C. After precontracting with a voltage (KCl)- or a receptor (5-hydroxytryptamine)-mediated agonist, cumulative concentration–relaxation curves were established for: pure succinylcholine; Quelicin® from multidose vials containing 20 mg/ml succinylcholine, 1.8 mg/ml methylparaben, and 0.2 mg/ml propylparaben; Anectine® from single-dose vials containing 20 mg/ml succinylcholine; multidose Anectine® containing 20 mg/ml succinylcholine and 1.0 mg/ml methylparaben; and methylparaben and propylparaben alone. When required, the endothelium of dog artery was removed by gentle mechanical rubbing and the response to the drugs reevaluated. Both Quelicin® and multidose Anectine® produced statistically significant (*P* < 0.05) relaxation; Quelicin® was the more potent of the two. Methylparaben and propylparaben produced relaxation in an additive manner and completely accounted for the relaxation produced by Quelicin® and multidose Anectine®. The vascular relaxation was found to be independent of the presence of a functional endothelium. Consistent with a nicotinic induced contraction, pure succinylcholine maintained vessel tone. It is concluded that the pharmaceutically ubiquitous preservatives methylparaben and propylparaben but not pure succinylcholine have vasoactive properties *in vitro*. (Key words: Artery: endothelium-derived relaxation factor; vascular smooth muscle. Neuromuscular relaxants: succinylcholine. Preservatives: methylparaben.)

SUCCINYLCHOLINE is a commonly used neuromuscular blocking agent. Its quick onset and short duration of action have made it the drug of choice when rapid tracheal intubation is needed. Unfortunately, succinylcholine has been reported to increase intracranial pressure (ICP) in cats,[1,2] dogs,[3] and humans[4-6], thus giving rise to controversy about its safety in patients with intracranial disease. Increases in ICP have been attributed to the acetylcholinelike, nicotinic, depolarizing actions of succinylcholine and may be related to the characteristic muscle fascicu-

lations or to afferent muscle spindle activity and subsequent EEG arousal with concomitant increases in cerebral blood flow (CBF) and volume.[3,4,6,7] Others have argued that succinylcholine's action on ICP may be associated with hemodynamic effects of tracheal intubation during light anesthesia.[8] Since these findings and hypotheses relate temporally to the period immediately after the intravenous administration of succinylcholine, it seemed logical to investigate a possible direct cerebrovascular effect of this drug. Others have reported a direct, albeit vasoconstricting, action of nicotine on dog basilar artery.[9] Accordingly, we investigated the activity of succinylcholine on basilar artery preparations from dog and guinea pig.

During these studies, it became apparent that a clinically available brand of succinylcholine, unlike samples prepared from the pure substance, was able to relax basilar arteries, suggesting that the preservatives present in the clinically available brand had pharmacologic activity. The preservatives, methylparaben and propylparaben, have been shown previously[10] to have smooth muscle relaxant activity on respiratory smooth muscle *in vitro*. The following study reports the observed *in vitro* actions of pure succinylcholine, preservative-containing and non-preservative-containing clinically used brands of succinylcholine, and the preservatives themselves on basilar arteries from the dog and guinea pig. The finding in recent years of a significant role of the endothelium in the determination of vascular muscle tone,[11] including that of cerebral vessels,[12,13] dictated additional studies to address the role of the endothelium in the observed vasodilation.

Materials and Methods

This protocol was approved by The University of Western Ontario Council on Animal Care. Vessels from adult mongrel dogs were obtained from animals anesthetized with sodium pentobarbital (30 mg/kg) for removal of the heart for other studies. Guinea pigs of either sex and weighing 300–600 g were decapitated, with care taken to avoid subarachnoid hemorrhage. After craniotomy, the brains were removed and placed in cold modified Krebs' solution (composition: 118.3 mM NaCl, 4.7 mM KCl, 1.2 mM KH$_2$PO$_4$, 1.2 mM MgSO$_4$, 22.1 mM NaHCO$_3$, 2.5 mM CaCl$_2$, 11.1 mM dextrose, and 0.026 mM calcium disodium EDTA) gassed with 5% CO$_2$–95% O$_2$. Under a binocular microscope, the basilar artery was dissected free from the arachnoid membrane and con-

nective tissue, with care taken not to damage the endo-thelium.

Using a method modified from Hogestatt et al.,[14] sections of basilar artery rings 4 mm (dog) or 1 mm (guinea pig) long were cut and suspended at minimal tension between two parallel stainless steel wires in 3 ml double-walled glass organ baths at 37°C. One of the wires was rigidly attached to an FT03 isometric force transducer (Grass Instrument Co., Quincy, MA), and the other to a micromanipulator (Marzhauser MM3, Fine Science Tools, North Vancouver, BC, Canada). After equilibrating for 60 min, the diameter was increased gradually in a step-wise manner by turning the micrometer or by applying a load over a pulley to the other side of the transducer. A resting tension of 2 g was used for the dog basilar artery, and a load of 100 mg was used for the guinea pig. This resulted in responses to agonists that were reproducible and consistent with our previous experience and that of others.

As in a previous study on dog arteries, the presence of a functional endothelium was established by the presence of an inhibitory response to 0.2 μM arginine vasopressin.[15] When required, the endothelium was mechanically removed from the dog artery by gently inserting a probe into the lumen with a turning motion, and validated by testing or retesting with vasopressin to show the absence of relaxation.

Agonists were added to the organ baths by micropi-pettes to produce the final organ bath concentrations. To verify unchanged tissue responses during the course of the experiment, each tissue was exposed to 25, 35, and 65 mM KCl at the beginning and to 25, 35, 65 and 125 mM KCl at the end. For studies involving the measurement of inhibitory–relaxant activity, tissues were precontracted with a submaximal concentration of a voltage-mediated agonist (KCl 65 mM) or a receptor-mediated agonist, 5-hydroxytryptamine creatinine sulfate (5HT, 0.2 μM), and the inhibitory agent (succinylcholine, methyl-paraben, or propylparaben) was added in an incremental manner to produce cumulative concentration–response curves. Since we found the potassium-contracted dog bas-ilar artery apparently more resistant to relaxation due to parabens, a concentration of 25 mM KCl instead of 65 mM was used. Control measurements were obtained at

the beginning and randomly throughout the experiment by the addition of agonist to the vessel without subsequent study-drug administration. In the case of 5HT, which elicited a phasic response followed by a tonic response, the inhibitory agents were not added until the tonic phase plateau had been established.

Agents used on vessels both with and without endo-thelium were: pure succinylcholine chloride containing no methylparabens or propylparabens (SUX); a clinically used brand of Anectine® from single-dose vials containing 20 mg/ml succinylcholine chloride (A-noMP); a clinically used brand of Quelicin® from multidose vials containing 20 mg/ml succinylcholine chloride, 1.8 mg/ml methyl-paraben, and 0.2 mg/ml propylparaben (Q); multidose Anectine® containing 20 mg/ml succinylcholine chloride and 1.0 mg/ml methylparaben (A+MP); and methylpar-aben parahydroxybenzoic acid methylester (MP) and propylparaben parahydroxybenzoic acid propylester (PP). Supplies of SUX, Anectine® (single- and multidose vials) and MP and PP as pure substances were provided by Bur-roughs Wellcome Canada Ltd. Q was obtained from Ab-bott Laboratories Ltd. Arginine vasopressin, 5HT, and an additional supply of SUX (pure substance for com-parison) were purchased from Sigma Chemical Company, St. Louis, MO.

After precontracting with either a voltage mediated agonist (KCl) or a receptor mediated agonist (5HT), con-centrations of SUX, A-noMP, A+MP, Q, MP, PP, or MP+PP were added to the bath. The compounds and mixtures were diluted so that each microliter contained the same concentration as would be found in 1 μl of the commonly dispensed 20 mg/ml succinylcholine solution. For example, 1 μl of MP would contain the same amount of MP as would 1 μl of a 20 mg/ml solution of Q but would contain no succinylcholine or PP. For comparison, tables 1 and 2 are provided to show the exact amounts and final organ bath concentrations of each of the con-stituent(s) added to the organ bath.

Data (mean ± SEM) are expressed as percentage of the agonist (KCl or 5HT) response after the addition of the inhibitory agent. An analysis of variance (ANOVA) for repeated measures was used to examine for differences in the effects of drug concentration on vessels, and an ANOVA for independent samples or an unpaired t test

TABLE 1. Concentrations of Constituents of Clinically Used Drugs

Agent	Succinylcholine		Methylparaben		Propylparaben	
	mg/ml	mM	mg/ml	mM	mg/ml	mM
Quelicin	20.0	55.4	1.8	11.6	0.2	1.1
Anectine multidose	20.0	55.4	1.0	6.4	0.0	0.0
Anectine single dose	20.0	55.4	0.0	0.0	0.0	0.0

TABLE 2. Drug Concentration ($\mu l/ml$) in Water Bath

	5 $\mu l/ml$			10 $\mu l/ml$			20 $\mu l/ml$			30 $\mu l/ml$			40 $\mu l/ml$		
	SUX	MP	PP	SUX	MP	PP	SUX	MP	PP	SUX	MP	PP	SUX	MP	PP
Q	90.3	8.1	0.9	180.6	16.2	1.8	361.2	32.4	3.6	541.8	48.6	5.4	722.4	64.8	7.2
SUX	90.3	0.0	0.0	180.6	0.0	0.0	361.2	0.0	0.0	541.8	0.0	0.0	722.4	0.0	0.0
A–noMP	90.3	0.0	0.0	180.6	0.0	0.0	361.2	0.0	0.0	541.8	0.0	0.0	722.4	0.0	0.0
A + MP	90.3	4.5	0.0	180.6	9.0	0.0	361.2	18.0	0.0	541.8	27.0	0.0	722.4	36.0	0.0
MP	0.0	8.1	0.0	0.0	16.2	0.0	0.0	32.4	0.0	0.0	48.6	0.0	0.0	64.8	0.0
PP	0.0	0.0	0.9	0.0	0.0	1.8	0.0	0.0	3.6	0.0	0.0	5.4	0.0	0.0	7.2
MP + PP	0.0	8.1	0.9	0.0	16.2	0.6	0.0	32.4	3.6	0.0	48.6	5.4	0.0	64.8	7.2

Q = quelicin; SUX = succinylcholine; A = anectine; MP = methylparaben; PP = propylparaben.

was used for differences between drug groups. The Student–Newman-Keuls test was used when appropriate. $P < 0.05$ was considered statistically significant.

Results

During a preliminary study of the potential nicotinelike actions of succinylcholine, it was observed that samples of SUX, up to millimolar amounts, had no statistically significant effects upon the contractions elicited by KCl or 5HT on either the dog or guinea pig basilar arteries (fig. 1). When a sample of Q was used instead of SUX, a concentration-dependent relaxation was observed (fig. 1). Subsequent studies on the dog basilar artery showed that the Q brand of succinylcholine reproducibly relaxed 5HT-contracted vessels (fig. 2). Since the only difference between the SUX and the Q product was the presence of MP (1.8 mg/ml) and PP (0.2 mg/ml) in the latter, a solution was prepared containing both MP and PP at these concentrations and this solution was tested. These results on the dog basilar artery precontracted with 5HT (0.2 micromolar) are summarized in figure 3a. The lines for

Q and for MP+PP can be superimposed, indicating that the total relaxant activity is due either to MP or PP, or to both. Accordingly, the effect of MP alone and of PP alone were tested on the dog artery precontracted with 5HT (Fig. 3b). Both of these paraben preservatives contributed in an additive manner to the relaxation induced by Q; each paraben alone produced relaxation less than that of the combination, and the effect of the combination was indistinguishable from the effect of Q. Results with guinea pig basilar artery were qualitatively similar.

Figure 4 shows the effects of the commercially available succinylcholine preparations. The vasodilation is in proportion to the amounts of parabens present. It may be noted that succinylcholine without preservative maintained a tone higher than that seen with saline-treated
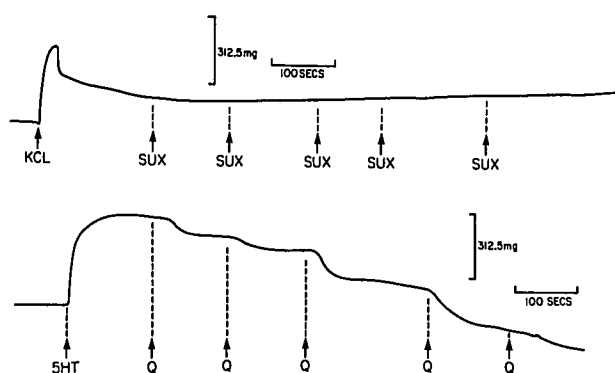

FIG. 1. (Top) The effect of cumulative concentrations of succinylcholine added to a basilar artery ring precontracted with 65 μM KCl. (Bottom) The effect of cumulative Quelicin concentrations added to a basilar artery ring precontracted with 0.2 μM 5 HT. SUX = succinylcholine without preservatives; Q = Quelicin (succinylcholine) with both methylparaben and propylparaben). See table 2 for concentrations.
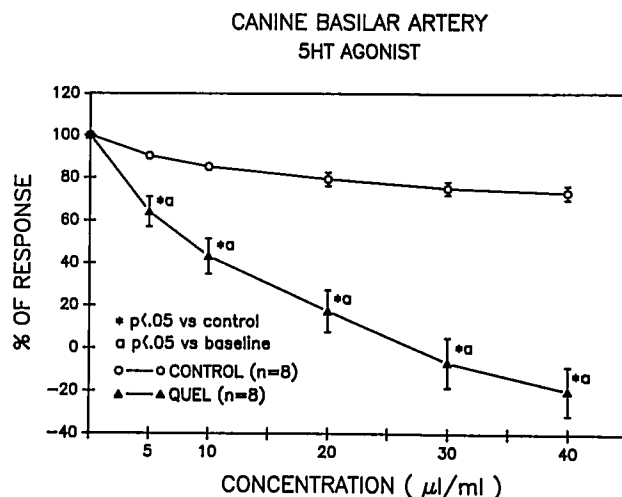

FIG. 2. The effect of cumulative concentrations of Quelicin added to canine basilar artery rings precontracted with 5 HT. Control vessels received equivalent volumes of saline. Zero percent is the resting tension, and 100% is the plateau tension after contraction with 5HT. Concentration = concentration of drug in waterbath (see table 2). Data are shown as mean ± SEM. Control = saline; SUX = succinylcholine without preservatives; ANEC + MP = Succinylcholine with only methylparaben; QUEL = succinylcholine with both methylparaben and propylparaben. a = $P < 0.05$ versus baseline; *$P < 0.05$ versus control.
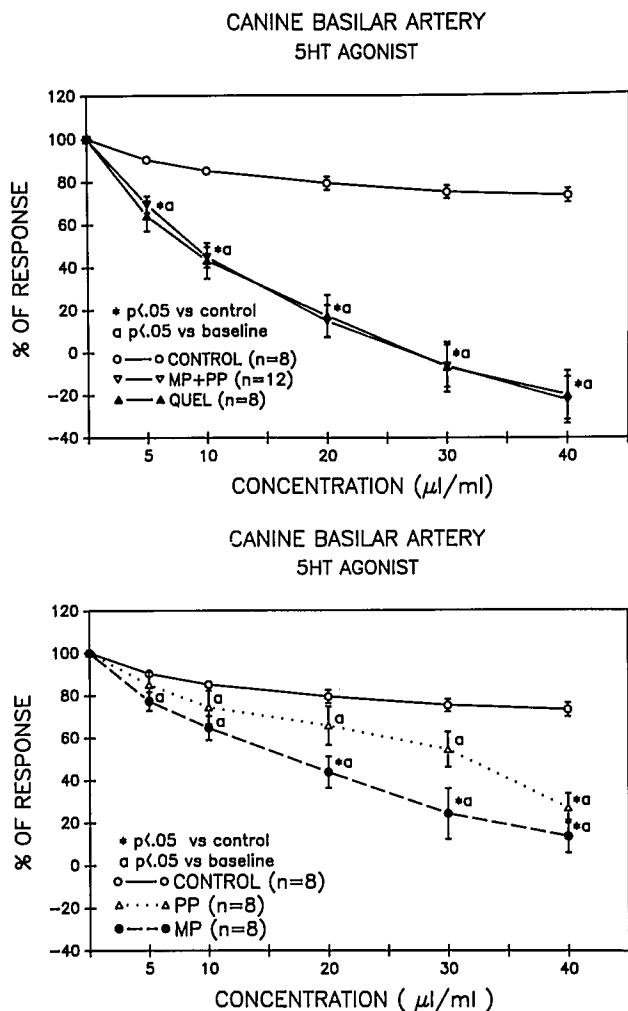
CANINE BASILAR ARTERY
5HT AGONIST

CONCENTRATION (μl/ml)

CANINE BASILAR ARTERY
5HT AGONIST

CONCENTRATION (μl/ml)

FIG. 3. (*Top*) The effect of cumulative concentrations of Quelicin and the equivalent concentrations of methylparaben and propylparaben contained in the Quelicin on canine basilar artery rings precontracted with 5 HT. Control vessels received equivalent volumes of saline. Zero percent is the resting tension, and 100% is the plateau tension after the contraction with 5HT. (*Bottom*) The effect of cumulative concentrations of propylparaben and methylparaben alone in concentrations identical to those used in the upper diagram. Data are shown as mean ± SEM. Concentration = concentration of drug in water bath (see table 2). Control = saline; MP = methylparaben; PP = propylparaben; QUEL = succinylcholine with both methylparaben and propylparaben. a = $P < 0.05$ *versus* baseline; *$P < 0.05$ *versus* control.

controls. Although the findings on the 5HT-precontracted vessels were not species-dependent, there was an apparent difference between the species when 65 mM KCl was used as the agonist: the KCl-precontracted dog basilar artery was more resistant to relaxation by these substances. However, when these experiments were repeated with 25 mM KCl as the agonist, the relaxation was found to be of similar magnitude to that in the guinea pig.

The role of the endothelium was determined in a series

of experiments using rubbed (endothelium mechanically removed) and unrubbed dog arteries exposed to all of the study substances. The observed relaxation was not dependent on the presence of a functional endothelium. Indeed, although not achieving statistical significance, the relaxation in the absence of endothelium tended always to be greater than that in the presence of endothelium. This effect was greater when succinylcholine was present.

Discussion

This study, in attempting to determine the presence, if any, of a direct nicotinic vascular action of the acetylcholinelike drug succinylcholine, serendipitously uncovered a marked relaxation due to the MP and PP preservatives. This relaxation was found to be reproducible *in vitro* and to be independent of the presence of a functional endothelium. Both MP and PP contributed in an additive manner to the relaxation due to Q, and it was the MP present in the multidose vials of Anectine® (A+MP) that produced its relaxation. On the contrary, SUX maintained tone, whereas the control response faded over time (fig. 4). The latter observation is consistent with a slight nicotinic-induced contraction due to SUX such as we predicted at the initiation of this study, based on the findings of Shirahase et al.[9]

After intravenous injection of paraben esters, they are cleared from the body within 6 h; appreciable amounts are transiently concentrated in the brain, spleen, and

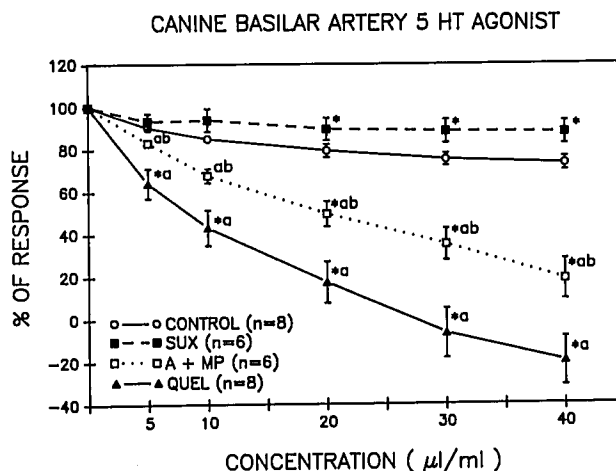### CANINE BASILAR ARTERY 5 HT AGONIST

CONCENTRATION (μl/ml)

FIG. 4. The cumulative concentration response of canine basilar artery rings precontracted with 5HT to commercially available succinylcholine preparations. Control = saline; SUX = succinylcholine without preservatives; A + MP = succinylcholine with only methylparaben; QUEL = succinylcholine with both methylparaben and propylparaben. a = $P < 0.05$ *versus* baseline; *$P < 0.05$ *versus* control; b = $P < 0.05$ A + MP *versus* QUEL. Data are shown as mean ± SEM. Concentration = concentration of drug in water bath (see table 2).

pancreas.[16] They are first hydrolyzed by esterases in the liver and kidney before excretion in the urine. Esterases in other tissues, including the brain and plasma, do not hydrolyze them. The inclusion of the parahydroxybenzoates as preservatives in intravenous solutions of a variety of drugs has previously been implicated in smooth muscle activity *in vitro* and *in vivo*.[10,17, 18]

The observation that vascular muscle was relaxed by the parabens raised the possibility that these parahydroxybenzoic acid esters were causing the release of a substance or substances from the endothelium. The existence of an endothelium-derived relaxing factor (EDRF) was first proposed in 1980 by Furchgott and Zawadzki,[11] who showed that the acetylcholine-induced relaxation of rabbit aorta required the presence of a functional endothelium. EDRF is able to act on the adjacent smooth muscle *via* a mechanism associated with the activation of guanylate cyclase and an increase in cyclic guanosine monophosphate (cGMP) concentration.[19-21] In the current investigation, it was apparent that removal of a functional endothelium as tested for by the addition of vasopressin did not remove the ability of the parabens to relax the muscle.[12] The site or sites of action of the parabens is therefore most likely to be directly upon the smooth muscle.

Previously,[10] we proposed that MP was a "nonspecific" spasmolytic. However, we did provide evidence from the guinea pig trachea to suggest an ability to specifically enhance the response to catecholamines but not to noncatecholamine bronchodilators. This, we proposed, may imply an action involving the inhibition of an extraneuronal catecholamine uptake mechanism.[22,23] The diversity of tissues on which we have shown the parabens to act— guinea pig trachea, rabbit jejunum, and now the dog and guinea pig basilar arteries—argues against a catecholamine-dependent mechanism(s) for all of the observations. Indeed, the canine basilar artery is notably resistant to catecholamines, and only at very high doses will it respond to catecholamines in an atypical manner by a slow contraction.[24]

On the basis of the lack of selectivity of the parabens, it is possible that their actions are related to their reported local anesthetic actions.[25,26] However, local anesthetic agents have recently been shown to produce endothelium-dependent vasodilation at a site distal to receptor activation at the endothelial cell and proximal to the smooth muscle guanylate cyclase mechanisms, an observation that would be inconsistent with our findings.[27] In contrast, others have reported diverse vascular actions of local anesthetics, which indicate that they may produce concentration-dependent vasoconstriction and relaxation.[28-31] A local anestheticlike action still may be consistent with our current findings.

The actual concentrations of succinylcholine, methylparaben, and propylparaben that reach the cerebral vasculature of patients is not known. However, we believe it is reasonable to assume that a drug such as succinylcholine, which is effective on its first pass through a tissue, is diluted initially in the central blood volume (heart and lungs, 500–1000 ml) and not in the entire blood volume. The concentrations reaching the brain would then be between 5 and 20 $\mu l/ml$ (see table 2 for actual concentrations). We propose that the use of these preservatives in intravenous solutions of some but not all commercially available brands of succinylcholine may contribute to the controversy regarding the effects of succinylcholine on CBF and ICP both in patients and in experimental animals. A review of the literature indicates that reports do not regularly include the source of the succinylcholine nor the presence or absence of parabens or other agents. The absence of this information makes it impossible to establish the role, if any, the preservatives may have played. However, in conscious volunteers, we have found that the intravenous administration of clinically relevant doses of methylparaben and propylparaben do not increase CBF (Gelb, unpublished data). Our proposal does not invalidate the findings regarding the prevention of CBF and ICP actions of succinylcholine by the prior administration of competitive neuromuscular blockers,[3,6,7] because to our knowledge the activity of these drugs on the cerebrovascular effects of parabens has not been investigated.

In conclusion, our finding that the pharmaceutically ubiquitous paraben preservatives have cerebrovascular dilating properties *in vitro* may need to be taken into consideration by future investigators and may encourage reinterpretation of previous studies.

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