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Isoflurane—A Study of Its Adrenoceptor Interaction in the Isolated Rat Parotid Gland

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Adrenergic receptor interaction with isoflurane was studied in an *in vitro* rat parotid gland model in which beta-adrenoceptor agonists evoke amylase release and alpha-adrenoceptor agonists induce potassium secretion from parotid cells. The amylase secretory studies were performed using a batch-incubation technique, and potassium efflux was evaluated using $^{86}Rb^+$ as a probe for K^+. Isoflurane was dissolved in a fat emulsion, which of its own had no secretory effect. Isoflurane induced a dose-dependent amylase release that was unaffected by beta-adrenergic blockade with propranolol and metoprolol. Isoflurane also induced a significant efflux of $^{86}Rb^+$ that could not be inhibited by the alpha-adrenoceptor antagonist, phentolamine. Dinitrophenol, an uncoupler of oxidative phosphorylation, had no effect on the isoflurane-induced enzyme release, indicating that amylase secretion occurred by passive leakage. It is suggested that isoflurane has no direct action on alpha- or beta-adrenoceptors. Isoflurane, however, induces potent cellular events that might be due to an unspecific effect on the cell membrane, thereby causing changes in membrane permeability. (Key words: Anesthetics, volatile: isoflurane. Receptors: adrenergic. Sympathetic nervous system: adrenergic receptors.)

ISOFLURANE PRODUCES a dose-dependent reduction in cardiac contractility.[1] When isoflurane is administered to healthy subjects, cardiac output is maintained by an increase in heart rate.[2,3] Isoflurane is also a potent systemic vasodilator.[4,5] The mechanisms by which isoflurane produces these effects are controversial. It has been suggested that stimulation of beta-adrenoceptors could be responsible for some of the hemodynamic effects, *e.g.*, tachycardia.[2,6] An alpha-adrenergic receptor blocking action by isoflurane could also explain the peripheral vasodilatory action of the compound. However, there are no solid data demonstrating that isoflurane has direct beta- and/or alpha-adrenergic activity. The aim of the present study was, therefore, to evaluate whether isoflurane acts directly on alpha- and/or beta-adrenoceptors. For this purpose, a well-characterized *in vitro* rat parotid gland model was used. With this model, the sympathetically evoked amylase release is mediated mainly by beta-adrenoceptors,[7,8] and the potassium efflux is mediated mainly by alpha-adrenoceptors.[9,10]

Materials and Methods

Tissue Preparation

Female Sprague-Dawley rats, 8 to 12 weeks old and weighing approximately 250 g were used in the study. The animals were fasted for 18 h but had free access to water. They were given a single intraperitoneal injection of pentobarbital (30 mg/kg), and the parotid glands were rapidly excised and immersed in basal medium as described in the following section. The glands were dissected free from connective tissue elements under a stereo microscope and cut to the appropriate size and number.

Secretory Studies of Amylase Release

A Krebs-Henseleit bicarbonate buffer supplemented with pyruvate, glutamate, and fumarate, as well as 1 g/l bovine serum albumin (BSA) and 0.6 g/l D-glucose, were used in the enzyme secretory studies. The medium was prewarmed to 37° C and equilibrated with 95% O_2 and 5% CO_2. Parotid tissue slices of 5 mg were transferred to incubation vessels and preincubated for 15 min in 500 μl of medium at 37° C in a metabolic shaker. After preincubation and a rinse in fresh buffer, 500 μl of prewarmed and gassed medium containing the different tested drugs was added. Isoflurane was dissolved in a fat emulsion (Intralipid®) and then added to the incubation medium to a concentration of $3 \cdot 10^{-8}$, $3 \cdot 10^{-6}$, $3 \cdot 10^{-5}$, or $3 \cdot 10^{-4}$ M. Control incubations, without added drugs and with only the fat emulsion present, were included in each set of experiments. The remaining tested drugs were: l-propranolol (10^{-5} M), metoprolol (10^{-5} M), and isoproterenol (10^{-6} M). The incubations lasted for 30 min, and the wet weights of the tissue samples were then recorded. The specimens were sonicated (Branson, Inc., 50 W 10 s) in a Na-K-phosphate buffer (50 mM, *p*H 6.9). In addition, dinitrophenol (10^{-4} M), an uncoupler of oxidative phosphorylation, was added to the incubation medium in order to evaluate whether the amylase secretion was an active energy-requiring process or merely an occurrence due to passive leakage.

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Amylase Assay

Incubation media and tissue homogenates were diluted with 50 mM phosphate buffer (*p*H 6.9) and assayed for amylase activity, using a micro-modification of the 3,5-dinitro-salicylate method with 2% starch as a substrate.[11] One unit of amylase was defined as the activity liberating reducing groups corresponding to one μmol of maltose monohydrate per min at 25° C. The amylase release was expressed as the percentage of amylase released into the medium in relation to the total amylase activity in media plus homogenate.

Secretory Studies of ^{86}Rb-efflux (potassium efflux)

Parotid pieces (1 mg total weight) were immersed in a HEPES (2-[*N*-hydroxyethylpiperazine-*N'*-yl]-ethanesulphonic acid)-buffered Krebs-Ringer solution (*p*H 7.40) complemented with fumarate, glutamate, pyruvate, and ascorbic acid. After preincubation for 30 min at 37° C, the pieces were incubated for 120 min in the presence of 28 μM ^{86}RbCl. They were then washed for 15 min in 5 ml nonradioactive basal medium and transferred to the incubation vessels containing 5 ml of basal medium with the different drugs added. The tested drugs were: isoflurane ($3 \cdot 10^{-5}$ M), phentolamine (10^{-4} M and $3 \cdot 10^{-6}$ M), and noradrenaline (10^{-6} M). In each set of experiments, control vessels with buffer and fat emulsion were included.

The incubations were carried out for 5 min, and the tissue pieces were transferred to aluminum foil and freed of surrounding fluid with a micropipette. They were then freeze-dried overnight (−40° C; 0.1 Pa), and the dry weight was determined on a balance.

Measurement of Radioactivity

Weighed parotid slices were dissolved in 100 μl Hyamine®, and the radioactivity was determined in a liquid scintillation spectrometer using Aquasol® as the scintillation medium. One milliliter from each test medium was also transferred to scintillation vials, and the radioactivity was measured. Triplicate samples of 5 μl from each labeling medium were used as external standards. The $^{86}Rb^+$-efflux was expressed as the percentage of $^{86}Rb^+$ released into the medium in relation to the total rubidium content in media plus pieces. The methods concerning the secretory studies have been described previously in detail.[7–9]

Chemicals

^{86}RbCl was from Radiochemical Centre, Amersham, Buckinghamshire, United Kingdom. Hyamine® (p-[diisobutyl-cresoxyethoxyethyl] dimethylbenzyl-ammonium hydroxide) was from Packard Instrument Company, Downers Grove, Illinois. L-noradrenaline bitartrate, L-isoproterenol, and HEPES were purchased from Sigma Chemical Company, St. Louis, Missouri. Metoprolol and l-propranolol were supplied by Hässle AB, Sweden, and phentolamine was supplied by Smith Kline & French Laboratories, Limited, Welwyn Garden City, United Kingdom. Soluble starch and 3,5-dinitrosalicylate were bought from E. Merck AG, Darmstadt, West Germany. Aquasol® was from NEN Chemicals GmbH, Dreieich, West Germany. All reagents were of analytical grade. Sterile, deionized water was used throughout the experiments.

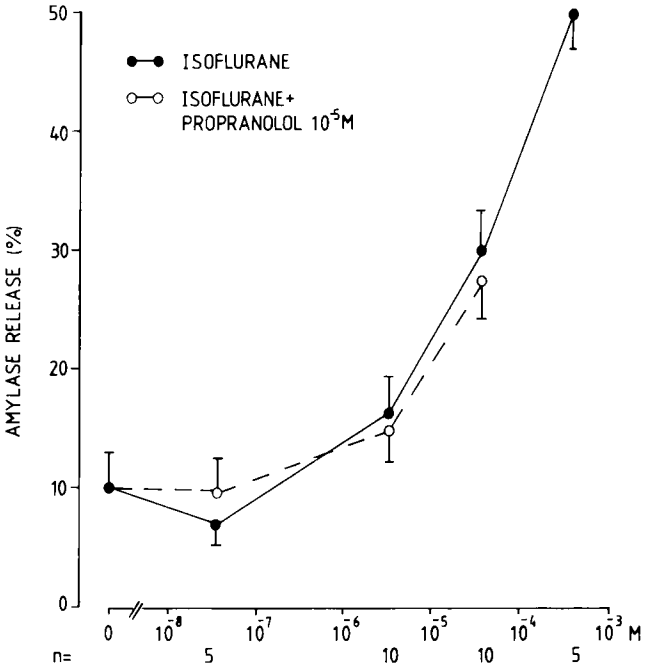

FIG. 1. Concentration–response curve for isoflurane with (*open circles*) and without (*closed circles*) l-propranolol (10^{-5} M). Amylase release is expressed as a percentage of amylase released into the medium in relation to the total amylase activity in medium plus homogenate. The values are presented as means ± SEM.

Statistical Methods

The two-tailed Student's *t* test and Wilcoxon rank sum test were used for the statistical analysis. A *P* value less than 0.05 was considered as statistically significant. Values are presented as means ± SEM.

Results

Amylase Release

Isoflurane caused a dose-dependent amylase discharge that was unaffected by the nonselective beta-antagonist propranolol (10^{-5} M) (figs. 1 and 2) and by the beta-1-selective antagonist metoprolol (10^{-5} M) (fig. 2). The beta-agonist isoproterenol was also a potent secretagogue, and

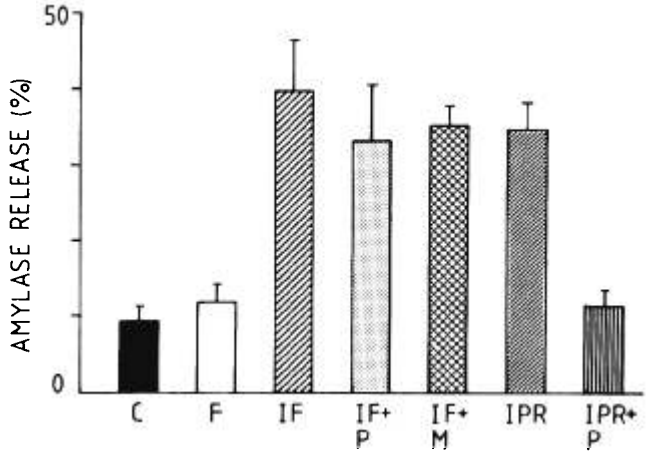

FIG. 2. Effects of listed drugs on amylase release from the rat parotid gland. C = control, nonstimulated basal amylase release; F = fat emulsion, incuding in the incubation medium; IF = isoflurane ($3 \cdot 10^{-5}$ M) dissolved in a fat emulsion and including in the basal medium; IF + P = isoflurane ($3 \cdot 10^{-5}$ M) and l-propranolol (10^{-5} M); IF + M = isoflurane ($3 \cdot 10^{-5}$ M) and l-metoprolol (10^{-5} M); IPR = isoproterenol (10^{-6} M); IPR + P = isoproterenol (10^{-6} M) and l-propranolol (10^{-5} M). Amylase release is expressed as a percentage of amylase released into the medium in relation to the total amylase activity in medium plus homogenate. The values are presented as means ± SEM for five separate experiments and animals.

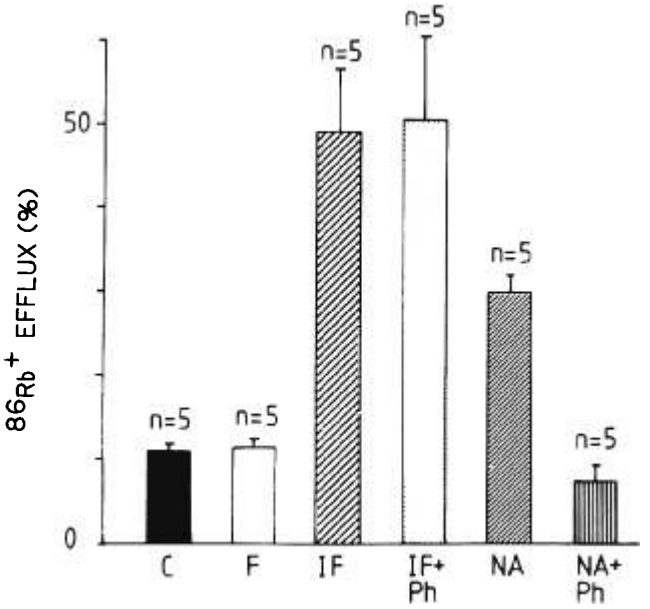

FIG. 3. Effects of listed drugs on $^{86}Rb^+$ efflux from the rat parotid gland. C = control, nonstimulated basal efflux; F = fat emulsion, including in the incubation medium; IF = isoflurane ($3 \cdot 10^{-5}$ M) dissolved in a fat emulsion and including in the basal medium; IF + Ph = isoflurane ($3 \cdot 10^{-5}$ M) and phentolamine (10^{-4} M); NA = noradrenaline (10^{-6} M); NA + Ph = noradrenaline (10^{-6} M) and phentolamine ($3 \cdot 10^{-6}$ M). $^{86}Rb^+$-efflux is expressed as a percentage of Rb released into the medium in relation to the total Rb activity in medium plus homogenate. The values are presented as means ± SEM for five separate experiments and animals.

this stimulation was clearly abolished by metoprolol and propranolol (fig. 2). The fat emulsion was, of its own, without enzyme secretory effect.

In another set of experiments, an initial isoflurane ($3 \cdot 10^{-5}$ M) stimulation was followed by a second stimulation with isoproterenol (10^{-6} M). A profound amylase discharge was still observed with isoproterenol (control = 10.9 ± 2.2% *vs.* isoproterenol = 33.5 ± 3.5%).

Rubidium (potassium) Efflux

Isoflurane ($3 \cdot 10^{-5}$ M) produced a significant efflux of $^{86}Rb^+$. The alpha-adrenoceptor blocking agent phentolamine was without inhibitory effect. In contrast, the noradrenaline stimulated efflux was clearly reduced by this alpha-blocker (fig. 3). The fat emulsion, of its own, did not modify the $^{86}Rb^+$-efflux.

Uncoupling of Oxidative Phosphorylation

Dinitrophenol (10^{-4} M) completely abolished the isoproterenol-induced amylase secretion, whereas the isoflurane-stimulated amylase release was not affected (fig. 4). There was no effect by dinitrophenol on basal secretion values.

Discussion

In the present study, we have used a well-characterized rat parotid gland model to test if isoflurane has any direct action on adrenergic receptors. Activation of the alpha-adrenoceptor mainly regulates the inorganic ion secretion, *e.g.*, potassium efflux.[9,10] The alpha-adrenoceptor primarily involved is considered to be of the alpha-1-subtype.[10] Prenalterol, which is a potent, highly selective beta-1-agonist in the sinus node, atrium, and ventricle of the cat myocardium,[12] is a potent stimulator of amylase release from the rat parotid gland.[7] Sympathetically evoked amylase release is mainly a beta-1-adrenoceptor event, and stimulation of beta-2-adrenoceptors is of minor importance.[8,13]

It has been suggested that isoflurane stimulates beta-adrenoceptors.[2,6,14] In 1975, Philbin and Lowenstein observed that systemic vascular resistance did not increase when propranolol was administered to dogs anesthetized with isoflurane.[15] They suggested that isoflurane might act on peripheral beta-adrenergic receptors. The authors could not confirm these results in a later study,[16] but found that systemic circulation was better preserved during profound beta blockade and deep isoflurane anesthesia than during deep halothane anesthesia. An increase in coronary venous noradrenaline concentration has been observed

in dogs administered high concentrations of isoflurane, and a cardiac sympathetic action by the compound was suggested.[17]

The published hemodynamic effects of isoflurane in humans and dogs indicate only indirectly that the anesthetic agent might have a beta-agonistic action. There is no previous investigation demonstrating direct adrenoceptor interaction by isoflurane.

In our study, isoflurane was dissolved in a fat emulsion (Intralipid®) before being added to the Krebs solution. This was necessary because isoflurane is a highly lipid-soluble compound. The fat emulsion, of its own, had no effect on the cellular events studied.

We found that isoflurane induced a pronounced and dose-dependent amylase release from the rat parotid gland. This was not mediated through stimulation of beta-adrenoceptors because the beta-adrenoceptor antagonists propranolol and metoprolol could not block the secretory response. In comparison, the isoproterenol-evoked enzyme release was antagonized by both propranolol and metoprolol. In contrast to the beta-adrenoceptor-induced secretion by isoproterenol, the high enzyme release caused by isoflurane in our study was unaffected by dinitrophenol, a blocker of the oxidative phosphorylation. This indicates that the isoflurane-evoked amylase release is not related to energy-dependent processes, but rather depends on passive diffusion, probably by effects on cell-membrane components.

In order to evaluate if the pathways for amylase secretion were irreversibly affected by isoflurane, a second stimulation with isoproterenol following an initial isoflurane stimulation was performed. The parotid gland cells were still responsive to isoproterenol, indicating that the effect of isoflurane on the cell was reversible.

The potent vasodilatory effect of isoflurane could theoretically be due to a peripheral blockade of alpha-adrenoceptors. We could, however, not find any direct evidence of alpha-adrenoceptor action of the anesthetic. Isoflurane had a similar action on the Rb-efflux as the alpha-adrenoceptor agonist noradrenaline. The isoflurane-induced ion secretion was, however, not mediated by stimulation of alpha-adrenoceptors, as the alpha-adrenoceptor antagonist phentolamine could not inhibit this response. The stimulatory effects by isoflurane on amylase secretion and Rb-efflux may consequently be due to other cellular events. An unspecific increase in cyclic adenosine monophosphate (AMP), changes in intracellular *p*H or the ratio between intracellular and extracellular calcium, or unspecific effects on membrane components by isoflurane may explain our results. The two secretory processes studied, exocytosis (amylase release) and electrolyte secretion (Rb-efflux), which are mediated *via* different pathways, were both influenced by isoflurane. This may suggest an unspecific action by the compound on the cell membrane.

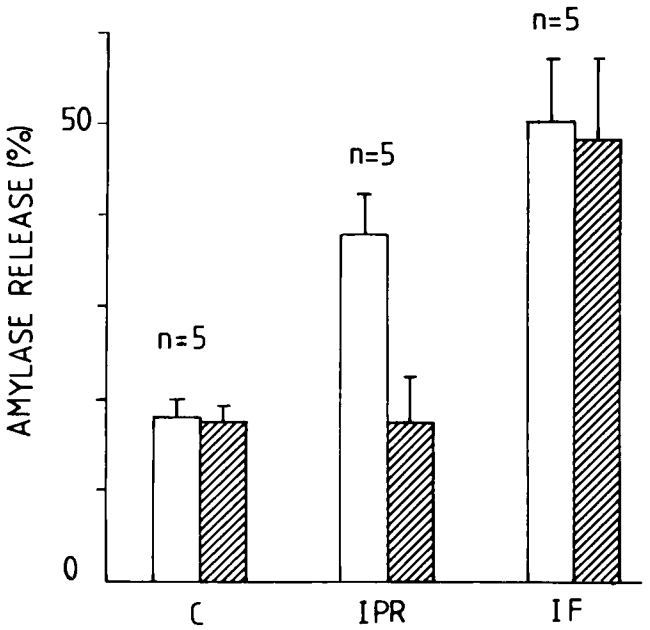

FIG. 4. Effect of dinitrophenol (DNP, 10^{-4} M) (*hatched columns*) on basal- (C), isoproterenol- (IPR, 10^{-6} M), and isoflurane- (IF, $3 \cdot 10^{-5}$ M) stimulated amylase release from incubated rat parotid gland. Amylase release is expressed as a percentage of amylase released into the medium in relation to the total amylase activity in medium plus homogenate. The values are presented as means ± SEM for five separate experiments and animals.

In conclusion, we suggest that isoflurane has no direct effect on alpha- or beta-adrenoceptors. However, isoflurane dose-dependently induces potent cellular events, which might be due to an unspecific effect, *e.g.*, on cell membrane components, thereby causing changes in membrane permeability.

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