THE EFFECTS OF KETAMINE, PHENCYCLIDINE AND LIDOCAINE ON CATECHOLAMINE SECRETION FROM CULTURED BOVINE ADRENAL CHROMAFFIN CELLS

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Summary

The ability of ketamine, phencyclidine and analogues to alter catecholamine secretion from cultured bovine adrenal chromaffin cells was investigated. Both ketamine and phencyclidine specifically inhibited nicotinic agonist-induced secretion at concentrations which did not alter secretion induced by elevated K⁺ depolarization. The inhibition of nicotinic agonist-induced secretion was not overcome by increasing concentrations of nicotinic agonist. The effects of stereoisomer pairs of phencyclidine-like drugs - dexoxadrol, levoxadrol and (+)PCMP, (-)PCMP - did not reveal stereospecificity for the inhibition, in contrast to the stereospecific behavioral effects of the drugs. The local anesthetic lidocaine (0.3 mM) also noncompetitively inhibited nicotinic agonist-induced secretion without inhibiting elevated K⁺-induced secretion. The data indicate that ketamine and phencyclidine at clinically relevant concentrations specifically inhibit the adrenal chromaffin cell nicotinic receptor at a site similar to or identical with the site of action of local anesthetic. Although the nicotinic receptor inhibition is probably not related to the anesthetic and behavioral effects of ketamine and phencyclidine, it is likely that the centrally mediated increase in sympathetic nervous system activity which is characteristic of these drugs is moderated by the peripheral blocking effects on catecholamine secretion from the adrenal medulla.

Ketamine is a widely used intravenous general anesthetic with significant behavioral side effects (1). The related compound phencyclidine is also a general anesthetic but is not used clinically because of its more intense behavioral effect which produces a schizophrenia-like syndrome in man (2). Phencyclidine ("Angel Dust") is a widely used drug of abuse and its pharmacology is of great clinical importance. These compounds alter the function of various ionic channels in excitable tissue and inhibit the function of nicotinic receptors on skeletal muscle (3). We have investigated the effects of

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Abbreviations: DMPP, 1,1 dimethyl-4-phenylpiperazinium; PCMP, 1-(1-phenylcyclohexyl)-3-methylpiperidine; PSS, Physiological salt solution.
ketamine, phencyclidine and analogues on catecholamine secretion from cultured bovine adrenal chromaffin cells. We found that the drugs specifically and noncompetitively inhibited secretion induced by nicotinic agonists in a manner similar to the inhibition caused by the local anesthetic lidocaine. The concentrations of drugs which inhibit secretion from chromaffin cells indicate that clinically the drugs may inhibit the physiological response of the adrenal medulla. While this work was in progress, Malave et al. reported that phencyclidine inhibited acetylcholine-induced secretion from perfused bovine adrenal glands (4).

Materials and Methods

Cells disaggregated from bovine adrenal medullae were added to 16 mm diameter uncoated culture wells (Costar, Cambridge, Mass.) at a density of 500,000 cells/well in 1 ml of Eagle's MEM (GIBCO, Grand Island, N.Y.) supplemented with 10% heat-inactivated fetal calf serum (GIBCO), 10 μM cytosine arabinoside (to inhibit fibroblast proliferation), gentamycin (50 μg/ml), and Fungizone (2.5 μg/ml) (Squibb, Princeton, N.J.) (5). After 4 days at 34°C in 5% CO₂/95% air the chromaffin cells had formed monolayers which contained approximately 20 nmoles catecholamine per well. The incubation medium was replaced after 4 days and experiments were performed on days 5-10. Medium was replaced with cytosine arabinoside-free medium 24 hours before an experiment. Experiments were performed at 25°C in physiological salt solution containing 145 mM NaCl, 5.6 mM KCl, 2.2 mM CaCl₂, 0.5 mM MgCl₂, 5.6 mM glucose, 15 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.4), and 0.5 mM ascorbic acid. In most experiments, 0.5% bovine serum albumin was also present. Elevated K⁺ PSS contained 56.0 mM KCl and 95.0 mM NaCl. The release of endogenous catecholamine was measured using a fluorescent assay as previously described (5). Secretion was measured after 15 minutes in the presence or absence of various drugs. Secretion is expressed as the fraction of the total catecholamine released into the medium and the data presented as mean ± standard error of the mean. Statistical significance was evaluated with Student's 't' test. Ketamine, phencyclidine and analogues were kindly provided by Dr. James Woods, Department of Pharmacology, University of Michigan Medical School.

Results

Effects of phencyclidine and ketamine on secretion. Both phencyclidine and ketamine caused a dose-dependent inhibition of secretion stimulated by the nicotinic agonist DMPP (Figure 1). The inhibition of both compounds was virtually complete at the highest concentrations investigated. Phencyclidine was approximately 7 times more potent than ketamine.

Although phencyclidine was a potent inhibitor of nicotinic agonist-induced secretion, it had no effect on secretion induced by depolarization with elevated K⁺-containing solution (Figure 2) or by Ba²⁺ substitution for Ca²⁺ for 12 minutes (data not shown). Similar results were obtained with ketamine (data not shown). Secretion induced by elevated K⁺ is probably initiated by Ca²⁺ entering through voltage sensitive Ca channels and does not involve the nicotinic receptor (5). Secretion induced by Ba²⁺ also does not involve stimulation of the nicotinic receptor. The data, therefore, indicate that both phencyclidine and ketamine block secretion by specifically inhibiting the nicotinic receptor-channel complex and not by inhibiting events within the cell after nicotinic receptor-channel activation.

Characterization of inhibition, reversibility, and stereospecificity. The ability of increasing concentrations of DMPP to overcome the phencyclidine-
Ketamine and phencyclidine inhibition of nicotinic agonist-induced catecholamine secretion. Chromaffin cells were incubated in the presence or absence of the nicotinic agonist DMPP (10 μM) together with various concentrations of either phencyclidine (PCP) or ketamine. After 15 minutes, the amount of DMPP-induced secretion was determined. There were 4 wells/group. DMPP-induced secretion in the absence of inhibitor was 25% of the total catecholamine and corresponds to 100 in the figure. Basal release in the absence of DMPP was 3% and was unaltered by phencyclidine or ketamine.

Effects of phencyclidine on DMPP-induced and elevated K⁺-induced catecholamine secretion. Chromaffin cells were incubated in the presence or absence of DMPP (10 μM) or elevated K⁺ (56 μM). Phencyclidine (PCP, 10 μM) was present where indicated. Secretion was determined after 15 minutes. There were 4 wells/group. *p < 0.001 vs. no drug.
induced inhibition of secretion was investigated (Figure 3). The blockade of secretion by a moderate concentration of phencyclidine was not surmountable by increased concentrations of DMPP. These data suggest that phencyclidine was not competing with DMPP at the receptor binding site. However, the effect of phencyclidine was reversible. Incubation of cells with 10 μM phencyclidine for 10 minutes followed by a 10 minute incubation in phencyclidine-free solution resulted in subsequent DMPP-induced secretion that was within 90% of DMPP-induced secretion from cells not incubated with phencyclidine.

The effects of phencyclidine on the concentration dependency of DMPP-stimulated secretion. Chromaffin cells were incubated in varying concentrations of DMPP in the presence or absence of phencyclidine (PCP), 4 μM. Secretion was determined after 15 minutes. There were 4 wells/group.

The IC₅₀'s for 2 pairs of stereoisomers of phencyclidine-like compounds - dexoxadrol, levoxadrol, and (+)PCMP, (-)PCMP - were investigated (Table 1). There was little difference in the potency in either pair of stereoisomers.

Effects of the local anesthetic lidocaine on catecholamine secretion. Local anesthetics are thought to inhibit the nicotinic receptor-channel complex by blocking ion flux through the channel and not by interfering with the agonist binding sites (6). The effects of the local anesthetic lidocaine on secretion from chromaffin were compared to those of phencyclidine. Lidocaine (0.3 mM) inhibited DMPP (10 μM)-induced catecholamine secretion 70% but did not inhibit elevated K⁺-induced secretion (Figure 4A) or Ba²⁺-induced secretion (data not shown). The data are consistent with the selective inhibition by the local anesthetic tetracaine of acetylcholine-induced catecholamine secretion from suspended chromaffin cells (7). The blockade induced by lidocaine was not surmountable by increasing concentrations of DMPP (Figure 4B). Thus, both phencyclidine and lidocaine inhibit secretion by interacting with the nicotinic receptor-channel complex at a site that is probably different from the site of interaction of nicotinic agonists.
TABLE 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;</th>
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<tbody>
<tr>
<td>Phencyclidine</td>
<td>3 x 10&lt;sup&gt;-6&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ketamine</td>
<td>2 x 10&lt;sup&gt;-6&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dexoxadrol</td>
<td>6 x 10&lt;sup&gt;-6&lt;/sup&gt;</td>
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<tr>
<td>Levoxadrol</td>
<td>4 x 10&lt;sup&gt;-6&lt;/sup&gt;</td>
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<tr>
<td>(+)PCMP</td>
<td>3 x 10&lt;sup&gt;-6&lt;/sup&gt;</td>
</tr>
<tr>
<td>(-)PCMP</td>
<td>4 x 10&lt;sup&gt;-6&lt;/sup&gt;</td>
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The ability of various concentrations of the above drugs to inhibit DMPP (10 μM)-induced secretion during a 15 minute incubation was determined. The IC<sub>50</sub>'s were estimated graphically and correspond to that concentrations of drug which inhibited DMPP-induced secretion 50%.

FIG. 4

Effects of lidocaine on catecholamine secretion. A) Catecholamine secretion stimulated by DMPP (10 μM) or elevated K<sup>+</sup> (56 mM) was determined after a 15 minute incubation in the presence or absence of lidocaine (Lid., 0.3 mM). B) Catecholamine secretion was determined after 15 minutes in various concentrations of DMPP in the presence or absence of lidocaine (0.2 mM). There were 4 samples/group. p <0.001 vs. no drug.

Discussion

Sites of action of ketamine, phencyclidine, and lidocaine. The general anesthetics and behaviorally active drugs ketamine and phencyclidine and the local anesthetic lidocaine inhibited nicotinic agonist-induced but not elevated K<sup>+</sup>-induced catecholamine secretion from adrenal chromaffin cells. The
inhibition by phencyclidine and lidocaine was not overcome by increased concentrations of nicotinic agonist. The data indicate that phencyclidine and ketamine inhibited secretion from bovine adrenal chromaffin cells by interaction with the nicotinic receptor-channel complex at a site that is probably different from the site of interaction of nicotinic agonist. The site may be similar to or identical with the site of interaction of lidocaine. Electrophysiological studies at the frog neuromuscular junction also demonstrated noncompetitive inhibition by phencyclidine of nicotinic stimulation (3). Binding of phencyclidine to the nicotinic receptor-channel complex of membranes from Torpedo marmorata indicates that there is a highly specific site of interaction which is probably not identical to the receptor binding site (8). Similar noncompetitive inhibition is caused by cholinergic antagonists such as hexamethonium, decamethonium, and histrionicotoxin on bovine adrenal chromaffin cells (9), on autonomic ganglion cells (10), and at the neuromuscular junction (11,12).

Studies on perfused bovine adrenal medulla resulted in similar but not identical findings to those in the present study. In perfused adrenal medulla phencyclidine (3 μM) inhibited acetylcholine-induced catecholamine secretion and caused little inhibition of the initial rate of Ba²⁺-induced secretion. However, phencyclidine significantly inhibited Ba²⁺-induced secretion after 3 minutes (4). Lidocaine which had little or no effect on elevated K⁺ or Ba²⁺-induced secretion in the present study (0.2 mM lidocaine), enhanced Ba²⁺-induced secretion from perfused bovine adrenal glands (0.1 mM lidocaine, 13). It is possible that cultured chromaffin cells have somewhat different pharmacological characteristics from chromaffin cells in perfused glands. However, it is also possible that phencyclidine and lidocaine in combination with Ba²⁺ alter catecholamine release into the perfusate because of indirect actions. For example, changes in the perfusion of the gland caused by changes in the vasculature may have been responsible for the effects of phencyclidine and lidocaine on Ba²⁺-induced secretion.

Relationship of inhibition of secretion from chromaffin cells by phencyclidine and ketamine to behavioral and clinical effects. The inhibition of DMPP-induced catecholamine secretion from chromaffin cells was not sensitive to differences in stereoisomers of phencyclidine-like drugs - dexoxadrol, levoxadrol, and (+)-PCMP (-)-PCMP. In behavioral experiments dexoxadrol is much more potent than levoxadrol (14) and has a 40-fold greater affinity for CNS receptors (15). (+)-PCMP is 5-6 fold more potent that (-)-PCMP in causing phencyclidine-like CNS effects (16). Therefore, the effects of phencyclidine on the nicotinic receptor-channel complex of bovine adrenal chromaffin cells are probably unrelated to interactions in the central nervous system which are responsible for the behavioral effects of phencyclidine. The effects of phencyclidine on activation of nicotinic receptors at the neuromuscular junction are also unrelated to the behavioral effects of phencyclidine (3). There is recent evidence that the behavioral effects of phencyclidine are correlated to the inhibition of a particular class of K⁺ channels (17).

Inhibition of nicotinic agonist-induced secretion from chromaffin cells by ketamine (IC₅₀ = 20 μM) and by phencyclidine (IC₅₀ = 3 μM) may be of clinical importance in ketamine-induced anesthesia and in drug abuse of phencyclidine. Concentrations of ketamine between 20 μM and 100 μM are attained during anesthesia (18). Concentrations of phencyclidine during acute intoxication can be as high as 2 μM (2). Both ketamine and phencyclidine characteristically activate peripheral sympathetic responses because of increased CNS stimulation of the peripheral sympathetic nervous systems (19). The inhibitory effects of ketamine and phencyclidine on nicotinic effects in the adrenal medulla probably moderates this increased stimulation.
Acknowledgements

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References