



Title	Mechanisms of catecholamine secretion and elevation of intracellular Ca <sup>2+</sup> concentration induced by muscarine and purine receptor in adrenal chromaffin cells of guinea pig
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base cation radicals.

The present study demonstrated that  $\cdot\text{OH}$  and  $e^-$  (or  $\cdot\text{H}$ ) were produced not only in bulk water but also in the hydration layer of DNA (DNA-bound water). However, though  $\cdot\text{OH}$  generated in the hydration layer could produce base damage such as 8-OHdG, they could not produce DNA strand breaks. Since scavenging of  $e^-$  (or  $\cdot\text{H}$ ) generated in the hydration layer

resulted in an increase of alkali-labile sites, these reactive species might negatively participate in the formation of alkali-labile sites. These results concerning the reactivities of  $\cdot\text{OH}$  as well as  $e^-$  (or  $\cdot\text{H}$ ) in the hydration layer will be helpful for understanding the mechanisms of radiation-induced damage in DNA in an intact environment with a small content of free water.

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### Mechanisms of catecholamine secretion and elevation of intracellular $\text{Ca}^{2+}$ concentration induced by muscarine and purine receptor in adrenal chromaffin cells of guinea pig

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Effects on catecholamine secretion and intracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) of muscarine and purine receptor activation were examined using perfused adrenal glands and dispersed chromaffin cells of the guinea pig.

1. Muscarine (1~300  $\mu\text{M}$ ) and pilocarpine (10~300  $\mu\text{M}$ ) caused a dose-dependent increase in catecholamine secretion from perfused adrenal glands. Muscarine was much more effective than pilocarpine. In dispersed adrenal chromaffin cells, all muscarinic agonists tested caused increases in catecholamine secretion in a dose-dependent manner. Muscarine and methacholine were more effective than bethanechol, oxotremorine and pilocarpine.

2. Muscarine caused a small increase in catecholamine secretion even in the absence of extracellular  $\text{Ca}^{2+}$  in both perfused adrenal glands and dispersed chromaffin cells.

3. Both 4-DAMP (0.1  $\mu\text{M}$ ) and pirenzepine

(0.1  $\mu\text{M}$ ), but not methoctramine (0.1  $\mu\text{M}$ ), shifted the dose-response curve for muscarine-induced catecholamine secretion to the right and inhibited increase in  $[\text{Ca}^{2+}]_i$ .

4. ATP (2~10 mM) caused a dose-dependent increase in catecholamine secretion from perfused adrenal glands. ADP, but neither AMP nor adenosine, was also effective in increasing catecholamine secretion, though its potency was much less than that of ATP.

5. ATP-induced secretory response was also observed under  $\text{Na}^+$  deficient conditions, but was reversibly abolished by removal of extracellular  $\text{Ca}^{2+}$ . In dispersed chromaffin cells, ATP (500  $\mu\text{M}$ ) caused increases in catecholamine secretion and  $[\text{Ca}^{2+}]_i$ , both of which were abolished after the removal of extracellular  $\text{Ca}^{2+}$ .

6. In fura-2 loaded-single chromaffin cells, KCl (40 mM), nicotine (100  $\mu\text{M}$ ) and muscarine (100  $\mu\text{M}$ ), all caused a rapid increase in  $[\text{Ca}^{2+}]_i$

that was maintained during their presence, though its magnitude gradually declined with time. The sustained  $[Ca^{2+}]_i$  rise induced by these drugs was reversibly blocked by exposure to a  $Ca^{2+}$ -free solution containing EGTA (500  $\mu$ M). When applied 30 s after removal of external  $Ca^{2+}$ , muscarine, but neither nicotine nor KCl, caused an early rise in  $[Ca^{2+}]_i$  transiently.

7. Muscarine-induced  $[Ca^{2+}]_i$  rise in  $Ca^{2+}$ -free solution was partially reduced by proceeding application of caffeine or by treatment with ryanodine plus caffeine.

8. Intracellular application of inositol 1, 4, 5-trisphosphate caused a transient increase in  $[Ca^{2+}]_i$  and inhibited the following  $[Ca^{2+}]_i$  response to muscarine without affecting the responses to nicotine and a depolarizing pulse.

9. Muscarine evoked membrane depolarization following a transient depolarization and brief hyperpolarization in most cells tested. There was a significant positive correlation between the amplitude of the depolarization and the magnitude of the sustained rise in  $[Ca^{2+}]_i$ . Muscarine-

induced sustained  $[Ca^{2+}]_i$  rise was much greater in the current-clamp mode than that in the voltage-clamp mode.

10. The sustained  $[Ca^{2+}]_i$  rise and  $Mn^{2+}$  influx in response to muscarine were suppressed by a voltage-dependent  $Ca^{2+}$  channel blocker, methoxyverapamil.

These results are summarized as follows : 1) catecholamine secretion and  $[Ca^{2+}]_i$  rise induced by muscarinic agonists may be mediated through  $M_1$ , or  $M_1$  and  $M_3$  muscarinic receptor subtypes in adrenal chromaffin cells of the guinea pig. 2) muscarinic receptor activation causes not only extracellular  $Ca^{2+}$  entry, but also  $Ca^{2+}$  mobilization from inositol 1, 4, 5-trisphosphate-sensitive intracellular stores, which are suggested to overlap those sensitive to caffeine. 3) either voltage-dependent  $Ca^{2+}$  channels or non-selective cation channels may function as the  $Ca^{2+}$  entry pathways activated by muscarinic receptor in guinea pig adrenal chromaffin cells. 4) ATP causes increase in catecholamine secretion exclusively by increasing the entry of extracellular  $Ca^{2+}$ .

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### Study of antitumor immunity induced by neocarzinostatin derivative, zinostatin stimalamaer

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Zinostatin stimalamer (ZSS) is a new anti-cancer agent derived from neocarzinostatin (NCS) which is synthesized by conjugation of one molecule of NCS and two molecules of poly(styrene-co-maleic acid). ZSS exhibited potent

*in vitro* and *in vivo* antitumor activity in preclinical experiments, and clinical trials of the intra-arterial administration of ZSS with iodized oil on hepatocellular carcinoma showed potent anti-tumor activity.