Insulin Rapidly Increases Diacylglycerol by Activating De Novo Phosphatidic Acid Synthesis

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The mechanisms whereby insulin increases diacylglycerol in BC3H-1 myocytes were examined. When [^{3}H]arachidonate labeling of phospholipids was used as an indicator of phospholipase C activation, transient increases in [^{3}H]diacylglycerol were observed between 0.5 and 10 minutes after the onset of insulin treatment. With [^{3}H]glycerol labeling as an indicator of de novo phospholipid synthesis, [^{3}H]diacylglycerol was increased maximally at 1 minute and remained elevated for 20 minutes. [^{3}H]Glycerol-labeled diacylglycerol was largely derived directly from phosphatidic acid. Insulin increased de novo phosphatidic acid synthesis within 5 to 10 seconds; within 1 minute, this synthesis was 60 times greater than that of controls. Thus, the initial increase in diacylglycerol is due to both increased hydrolysis of phospholipids and a burst of de novo phosphatidic acid synthesis. After 5 to 10 minutes, de novo phosphatidic acid synthesis continues as a major source of diacylglycerol. Both phospholipid effects of insulin seem important for generating diacylglycerol and other phospholipid-derived intracellular signaling substances.

INSULIN INCREASES DIACYLGLYCEROL (DAG) content (*1, 2*) and protein kinase C activity (*3*) in BC3H-1 myocytes. This activation may stimulate glucose transport and other metabolic processes (*4*). Concurrent, rapid increases in the contents of phosphatidic acid (PA), phosphatidylinositol (PI), polyphosphoinositides (PPI), and other phospholipids (*1, 2, 5, 6*) suggest that the increase in DAG is largely due to de novo phospholipid synthesis, rather than phospholipase C activation and PI plus PPI hydrolysis, as insulin has little or no effect on inositol phosphate generation (*2, 7*). However, phospholipase C activation (*8, 9*), small transient increases in inositol phosphates (*10*), and hydrolysis of a PI glycan (*11*) have been observed with insulin treatment. Thus, DAG may be increased by two mechanisms, de novo phospholipid synthesis and phospholipase C activation. We evaluated this by comparing [^{3}H]glycerol and [^{3}H]arachidonate incorporation into DAG in BC3H-1 myocytes.

In experimental conditions modified from those used previously (*1, 2*) (myocytes were incubated in Dulbecco's phosphate-buffered saline instead of serum-supplemented culture medium), DAG content increased 65% within 1 minute of insulin treatment and remained elevated for 20 minutes (Fig. 1). After incubating myocytes with [^{3}H]arachidonic acid for 120 minutes before insulin treatment, 80 to 90% of the [^{3}H]arachidonate was incorporated into phospholipids and lipids, presumably by rapid deacylation and reacylation because newly synthesized PA is poorly labeled by [^{3}H]arachidonic acid (*12*). Subsequent addition of insulin provoked a two- to threefold increase in [^{3}H]DAG within 0.5 minutes and, after 10 minutes, [^{3}H]DAG decreased to 30 to 50% above control levels (Fig. 1). When cells were incubated with [^{3}H]glycerol for 120 minutes before insulin treatment, less than 1% of the [^{3}H]glycerol was incorporated into phospholipids and lipids. Subsequent addition of insulin provoked a fivefold increase in [^{3}H]DAG production within 1 minute and this was maintained for 20 minutes (Fig. 1). These differences in insulin-induced increases in [^{3}H]DAG after [^{3}H]arachidonate and [^{3}H]glycerol labeling suggest that insulin increases DAG by two or more mechanisms.

The concurrent insulin-induced changes in phospholipids after labeling cells with [^{3}H]arachidonate are shown in Fig. 2A. The increase in [^{3}H]DAG production at 0.5 to 1 minute was associated with small, statistically insignificant increases in labeling of PA and phosphatidylcholine-phosphatidylethanolamine (PC-PE) and no change in PI

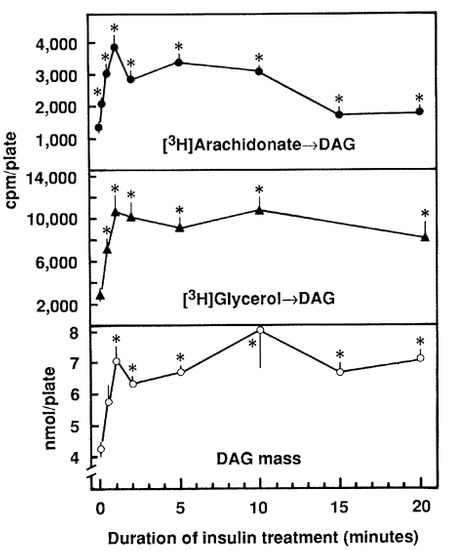

Fig. 1. Effects of insulin on DAG content and DAG labeling from [^{3}H]arachidonic acid and [^{3}H]glycerol in BC3H-1 myocytes. Myocytes were cultured for 10 to 14 days to confluence and functional maturity on 60-mm plates in Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% fetal calf serum (*1, 2*). Medium was removed and replaced with 3 ml of "DPBSGA" solution containing Dulbecco's phosphate-buffered saline, 0.1 m*M* $CaCl_2$, glucose (1 mg/ml), and bovine serum albumin (1 mg/ml). The cells were incubated for 120 minutes without (bottom panel) or with 15 μCi of 2-[^{3}H]glycerol (ICN; specific activity, 2 to 5 mCi/mmol) (middle panel) or 0.5 μCi of 5,6,8,11,12,14,15-[^{3}H](*N*)-arachidonic acid (NEN; specific activity, 238 Ci/mmol) (top panel). Albumin was deleted from the media in [^{3}H]arachidonate experiments. After 120 minutes of labeling, porcine insulin [200 n*M*, a maximally effective dose (*1, 2, 22*)] (Elanco) was added in a small volume of DPBSGA at varying times during a 20-minute treatment period. Control cells were also treated by addition of small volumes of DPBSGA during the 20-minute treatment period and this did not alter results. All cells were then incubated for 20 minutes, and the only variable was the duration of treatment with insulin. Reactions were stopped by addition of 3 ml of cold methanol. Plate contents were scraped and transferred to tubes containing 6 ml of chloroform. After mixing and phase separation, lipid extracts were washed three times with water, dried, and chromatographed on thin-layer plates by successive unidimensional development with benzene–diethyl ether–ethanol–acetic acid (50:40:2:2, by volume), followed by hexane–diethyl ether (94:6) (*23*). DAG areas were identified by iodine vapor staining and either assayed for radioactivity or eluted and assayed for DAG content. The latter was measured by incubating the dried DAG eluant for 5 minutes at 37°C in a DAG kinase assay system (*23*) containing 400 μl of H_2O, 250 m*M* sucrose; 50 m*M* potassium glycylglycine buffer (*p*H 6.5); 2.5 m*M* sodium deoxycholate, 2 m*M* $MgSO_4$, 1.5 μCi of γ-[^{32}P] adenosine triphosphate (ATP) (New England Nuclear), 2 m*M* ATP, and 100 μl of rat liver cytosol containing DAG kinase. The DAG kinase was obtained by homogenizing 1 g of rat liver in 5 ml of a solution containing 250 m*M* sucrose and 8 m*M* sodium deoxycholate, and centrifugation at 100,000*g* for 60 minutes. After incubation, [^{32}P]phosphatidic acid was purified by thin-layer chromatography (*1, 2, 23*), and DAG content was estimated by comparing results of samples to those observed with DAG standards. Shown here are mean ± SEM of four (bottom panel) or eight (upper two panels) determinations. ⋆, $P < 0.05$; determined by *t* test comparison of treatment groups to the control group, after establishing that there were significant differences between groups by analysis of variance (ANOVA).

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labeling. After 1 minute, there was nearly twice as much PI labeling; this increase may reflect rapid expansion of PI mass (Fig. 2B) (*1, 2*).

The transient insulin-induced increases in [^{3}H]DAG after [^{3}H]arachidonate labeling may have been due to phospholipase C–mediated hydrolysis of PI plus PPI (*8–10*), PC (*13*), or the PI glycan (*11*). Because insulin has little or no (*2*) effect on inositol phosphate generation in BC3H-1 myocytes, PI plus PPI hydrolysis cannot account for observed increases in [^{3}H]DAG. With respect to other sources of [^{3}H]DAG, [^{3}H]arachidonic acid was incorporated into PC and PE, but not appreciably into the chromatographic area (*11*) of the PI glycan. Similarly, incubation of myocytes with [^{3}H]myristic acid for 120 minutes resulted in strong (>90%) labeling of PC, less labeling of PE and PA, and no appreciable labeling of PI, PPI, or the PI glycan area; upon addition of insulin, [^{3}H]DAG increased threefold at 0.5 to 10 minutes and then diminished to control levels. These changes with [^{3}H]myristate were similar to those observed with [^{3}H]arachidonate labeling. Our findings suggest that, under the present experimental conditions, PC (± PE) is the most likely source of the [^{3}H]DAG generated by insulin treatment in [^{3}H]arachidonate- or [^{3}H]myristate-labeled myocytes. However, other sources, particularly as lesser contributors, cannot be excluded (*14*).

Insulin-induced changes in labeling of phospholipids and lipids after [^{3}H]glycerol labeling are shown in Fig. 2B. The initial increase in [^{3}H]DAG at 0.5 to 1 minute was associated with concomitant increases in the labeling of monoacylglycerol (MAG), triacylglycerol (TAG), PA, PI, and PC-PE (*15*); thus, insulin dramatically increased the rate of de novo PA and total glycerolipid synthesis during the first minute. After 1 minute, total glycerolipid labeling leveled off and labeling patterns of individual lipids varied considerably: after a twofold increase at 0.5 to 1 minute, [^{3}H]PA stabilized at 50% above the control level; [^{3}H]MAG and [^{3}H]TAG, like [^{3}H]DAG, remained elevated; [^{3}H]PC-PE diminished to control levels and increased later; [^{3}H]PI, after a twofold increase at 1 minute, decreased over the next 10 minutes and then increased again (*16*).

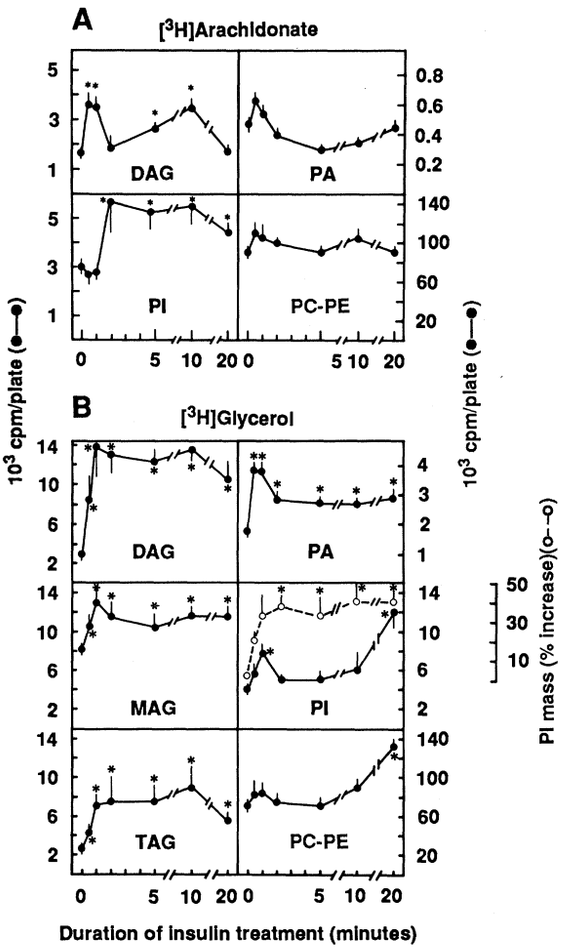

Fig. 2. Effects of insulin on (**A**) [^{3}H]arachidonate and (**B**) [^{3}H]glycerol incorporation into lipids and phospholipids in BC3H-1 myocytes. Experimental details were as in Fig. 1, except that the lipid extracts were split and analyzed for ^{3}H in DAG as in Fig. 1 and for ^{3}H in PA, PI, PC-PE, TAG, and MAG by thin-layer chromatography (*1, 2, 23*). Shown here are mean ± SEM of four determinations from a representative experiment. In experiments depicted by open circles and dashed lines in the PI panel, myocytes were incubated for 3 days with 10 μCi of [^{3}H]inositol (American Radiolabeled Chemicals; specific activity, 15 Ci/mmol) before the experiment to label inositol phospholipids to constant specific radioactivity (*2*); medium was removed, replaced with DPBSGA, and incubations were conducted as in other experiments; ^{3}H levels were used as a reflection of PI mass, and results are expressed as percent increase over control; shown here are mean ± SEM of four separate experiments, each conducted in quadruplicate. ⋆, $P < 0.05$ (ANOVA and t test).

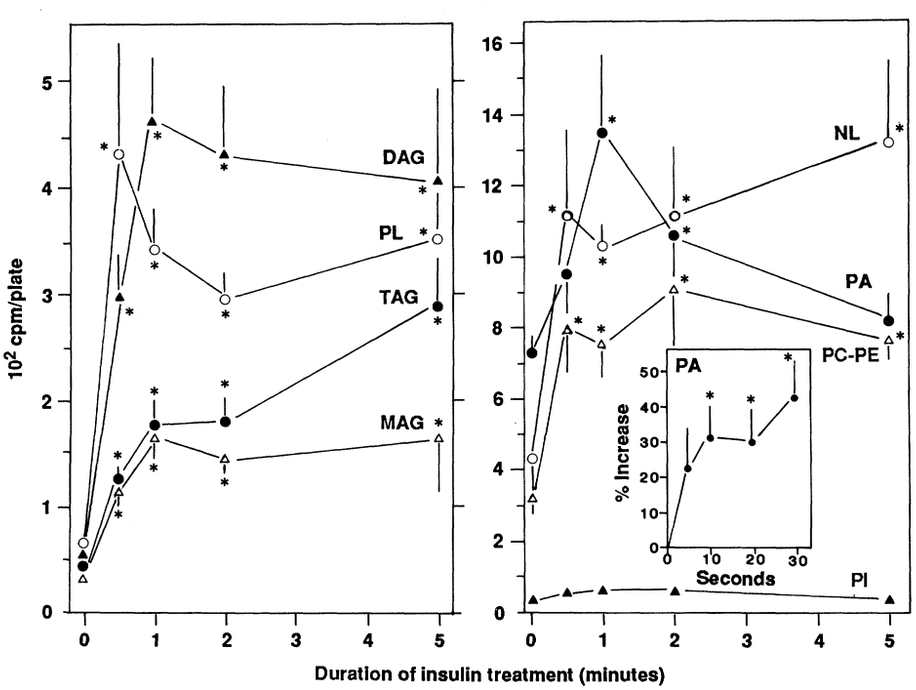

Fig. 3. Effects of insulin on [^{3}H]glycerol incorporation into phospholipids and neutral lipids after brief labeling. Experiments were identical to those of Fig. 2B, except that [^{3}H]glycerol was present for only 10 minutes before the 5-minute or 30-second (inset) experimental treatment period. During the treatment period, 200 n*M* insulin was present for the indicated times. Lipid extracts were analyzed either (**left**) for DAG, MAG, TAG, and total phospholipids (PL), or (**right**) in a separate experiment for PA, PI, PC-PE, and total neutral lipids (NL). Similar results were observed in other experiments. Shown here are mean ± SEM of 3 to 4 or 7 to 11 (inset) determinations. For the sake of simplicity, in the experiment shown on the left, error bars of controls are not shown, but were approximately 10% of the mean values. In the inset, PA data are expressed as the percent increase due to insulin treatment, to minimize differences in absolute PA values from three separate experiments. ⋆, $P < 0.05$ (ANOVA and t test).

Because all glycerolipids are derived from PA, total lipid-phospholipid labeling by [^{3}H]glycerol specifically reflects the rate of total de novo PA synthesis. After incubating cells for 120 minutes with [^{3}H]glycerol, subsequent addition of insulin for only 1 minute provoked a 50% increase in total [^{3}H]glycerolipids. Since labeling of glycerolipids by [^{3}H]glycerol progressed linearly during the 120-minute incubation prior to adding insulin, it was possible to estimate insulin-induced changes in the rate of total de novo PA synthesis; this rate increased 60-fold during the first minute of insulin treatment. This increase in total de novo PA synthesis was maximal at 20 to 200 n*M* and half-maximal at 2 n*M* insulin.

When myocytes were preincubated with [^{3}H]glycerol for 10 minutes instead of 120 minutes, insulin also provoked considerable increases in the labeling of PA, DAG, PC-PE, MAG, and TAG (Fig. 3). PI labeling was extremely small in these circumstances, and it appears that newly synthesized PA is preferentially metabolized directly to DAG, and thence to MAG, TAG, PC, and PE (*17*). Increases in [^{3}H]glycerol labeling of PA were evident at 5 to 10 seconds of insulin treatment, and [^{3}H]PA appears to be the major, if not sole, source of [^{3}H]DAG in these 10-minute labeling experiments.

When myocytes were labeled for either 10 or 120 minutes with [^{3}H]glycerol, subsequent treatment with insulin for 1 minute did not alter the labeling of tissue pools of free [^{3}H]glycerol or [^{3}H]glycerol-3′-phosphate, which were purified from aqueous delipidated extracts by anion exchange chromatography (*18*) [data summarized in (*16*)]. Phenylephrine, which potently and continuously activates phospholipase C and PI plus PPI hydrolytic turnover in BC3H-1 myocytes (*19, 20*), did not affect [^{3}H]glycerol incorporation into DAG or other lipids. Thus, insulin-induced increases in [^{3}H]glycerol incorporation into glycerolipids are not due to changes in either precursor labeling or PI plus PPI hydrolysis. In addition, de novo PA synthesis is not an obligatory concomitant of phospholipase C activation.

Our findings indicate that there is a burst of de novo PA synthesis during the first minute of insulin treatment, and this PA is directly converted to DAG and thence to PC and other lipids. Between 1 and 10 minutes of insulin treatment, the rate of de novo PA synthesis seems to subside somewhat [or may be masked by enhanced glycolysis (*16*)], and this appears to be a period of remodeling in which PI mass is increased and derived from a source labeled poorly with [^{3}H]glycerol but possibly well with [^{3}H]arachidonate (*21*). The source of this PI is uncertain, but it may be derived from hydrolysis of PC-PE. After 10 minutes of insulin treatment, de novo synthesis of PI and PC-PE appears to accelerate again.

Our findings further indicate that some agonists, such as insulin, simultaneously increase de novo PA synthesis while activating one or more types of phospholipase C. Whether these are parallel or sequential responses remains to be determined. In any event, these collaborative responses seem to be important for generating intracellular signaling substances. In the case of insulin, de novo PA synthesis appears to be important, both directly for DAG generation from PA and indirectly to provide substrate for phospholipase C–mediated hydrolysis of PC, the PI glycan or other phospholipids, with consequent generation of DAG and other phospholipid-derived intracellular signaling substances. Although precise quantitative contribution of each phospholipid effect remains to be determined, both probably contribute substantially to early increases in DAG (*16*), and de novo PA synthesis continues to be a major contributor at later times of insulin action.

Finally, it seems clear that insulin rapidly and massively increases PA synthesis by increasing glycerol-3′-phosphate acyltransferase activity. Moreover, this newly synthesized PA is preferentially converted directly to DAG. Since DAG activates protein kinase C, activation of de novo PA synthesis appears to provide a mechanism for activating protein kinase C without concurrently increasing inositol trisphosphate production and Ca^{2+} mobilization. This mechanism may be important in the action of insulin and other agents that increase de novo phospholipid synthesis.

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14. In accordance with the findings of Saltiel *et al.* [A. R. Saltiel, P. Sherline, J. A. Fox, *J. Biol. Chem.* **262**, 1116 (1987)], we have found that after labeling of myocytes for 20 hours with [^{3}H]myristate, there is a small, but definite, peak of radioactivity in the PI glycan and a larger peak in PI (the latter, however, is still very small in comparison to that of PC). Thus, particularly with longer labeling, some of the [^{3}H]DAG may be derived from the PI glycan, as well as PC. In the shorter 120-minute labeling experiments, however, there would have to be an extremely rapid turnover of [^{3}H]myristate in the PI glycan to explain our failure to observe radioactivity in this substance, as opposed to readily discernible labeling of DAG and PA. Although this is possible, the increases in [^{3}H]DAG observed with [^{3}H]arachidonate labeling cannot be due to hydrolysis of the PI glycan because, even after 20 hours, this substance is not measurably labeled. The possibility that de novo PA synthesis may also contribute to increases in [^{3}H]DAG after labeling myocytes with [^{3}H]arachidonate cannot be excluded, but it is unlikely because (i) [^{3}H]arachidonate is poorly incorporated into newly synthesized PA (*12*) and (ii) we were unable to observe significant insulin-induced increases in [^{3}H]PA after [^{3}H]arachidonate labeling.
15. As shown in other thin-layer chromatography systems, labeling of PC and PE accounted for approximately 80% and 20%, respectively, of the [^{3}H]glycerol found in the combined PC-PE areas.
16. We did not determine whether [^{3}H]glycerol-3′-phosphate–specific activity was decreased by insulin-induced increases in unlabeled glucose uptake, but glycerol-3′-phosphate levels may be increased by insulin (*18*), and the insulin effect on de novo PA synthesis may be underestimated, particularly after a lag of 1 minute (*22*). Along these lines, basal glycerol-3′-phosphate concentrations in rat adipose and heart tissues are approximately 0.25 m*M* (*18*). In 10-minute and 120-minute labeling experiments, ^{3}H labeling of glycerol-3′-phosphate (and derived glycolytic monophosphates) was approximately 3000 and 5100 cpm, respectively, per 60-mm plate (or approximately 7 μl of cellular water). If we assume that all of the radioactivity is in glycerol-3′-phosphate and that its concentration is 0.25 m*M*, it may be calculated from [^{3}H]DAG increases of 1,200 and 10,000 cpm per 60-mm plate in 10- and 120-minute experiments, that after 1 minute of insulin treatment approximately 1 to 3 nmol of DAG per 60-mm plate (or approximately 50% of the steady-state, insulin-induced increases in DAG content in Fig. 1) may have been derived from stimulation of de novo PA synthesis. This may be underestimated if, as is likely, some radioactivity is present in precursors other than glycerol-3′-phosphate or if insulin treatment results in glycerol-3′-phosphate concentrations in excess of 0.25 m*M*. Although future determination of the content and actual specific activity of glycerol-3′-phosphate in BC3H-1 myocytes will provide more precise quantitation, it seems likely that the de novo pathway contributes sizably to the observed increases in DAG content during insulin treatment. Moreover, because total glycerolipid labeling is increased approximately 50% after 1 minute of insulin treatment, and because virtually all of this is metabolized from PA through DAG to all other glycerolipids, it is clear that large quantities of DAG must be derived from the de novo pathway during insulin treatment.

    If we assume that PC hydrolysis is the major source of [^{3}H]DAG in experiments in which [^{3}H]arachidonic acid was used, it is also possible to estimate how much DAG may be derived by this mechanism. We have found that the content of PC in the BC3H-1 myocyte is approximately 75 nmol per 60-mm plate, as determined by measurement of phosphorus (*1*) in chromatographically purified PC. Because approximately 75% of the total [^{3}H]arachidonic acid in PC-PE is recovered in PC, the specific activity of PC in the experiment in Fig. 2A was approximately 1000 cpm/nmol. The increment in DAG after 1 minute of insulin treatment was approximately 1700 cpm, and this is equivalent to 1.7 nmol of PC or DAG. This estimate may be excessive if some of the increase in [^{3}H]DAG is derived from de novo PA synthesis, or if only part of the total PC pool is labeled. In any event, on the basis of these rough estimates, the increase in DAG that derives from de novo PA synthesis during the first minute of insulin treatment seems to be at least comparable in magnitude to the maximal estimates of that which may be derived from PC hydrolysis.
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21. Insulin-induced increases in labeling of PI by [^{3}H]arachidonate may be due to acylation or deacylation-reacylation of the rapidly expanded PI mass, or [^{3}H]arachidonate-labeled PC-PE may be converted to PI via DAG and PA by phospholipase C or phospholipase D action. Newly synthesized PA is poorly labeled by [^{3}H]arachidonic acid (*12*).
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Zinc Selectively Blocks the Action of *N*-Methyl-D-Aspartate on Cortical Neurons

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Large amounts of zinc are present in synaptic vesicles of mammalian central excitatory boutons and may be released during synaptic activity, but the functional significance of the metal for excitatory neurotransmission is currently unknown. Zinc (10 to 1000 micromolar) was found to have little intrinsic membrane effect on cortical neurons, but invariably produced a zinc concentration–dependent, rapid-onset, reversible, and selective attenuation of the membrane responses to *N*-methyl-D-aspartate, homocysteate, or quinolinate. In contrast, zinc generally potentiated the membrane responses to quisqualate or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate and often did not affect the response to kainate. Zinc also attenuated *N*-methyl-D-aspartate receptor–mediated neurotoxicity but not quisqualate or kainate neurotoxicity. The ability of zinc to specifically modulate postsynaptic neuronal responses to excitatory amino acid transmitters, reducing *N*-methyl-D-aspartate receptor–mediated excitation while often increasing quisqualate receptor–mediated excitation, is proposed to underlie its normal function at central excitatory synapses and furthermore could be relevant to neuronal cell loss in certain disease states.

A GROWING BODY OF EVIDENCE SUGgests that the transition metal zinc, a nutritive requirement with a widespread essential role in both plant and animal metabolism (*1*), may serve a specific signaling function in mammalian central excitatory neurotransmission. Chelatable Zn is present in high concentrations throughout mammalian brain, particularly in neocortical gray matter, pineal, and hippocampus (*2*). Ultrastructural studies have suggested that forebrain Zn is localized to synaptic vesicles in excitatory boutons (*3*). In the hippocampus, Zn is specifically localized in the terminals of the excitatory mossy fiber projection (*4*). Endogenous Zn is spontaneously released into the synaptic cleft (*5*), and a calcium-dependent increase in rate of Zn release can be evoked by electrical stimulation (*6*) or exposure to large amounts of potassium (*7*).

The hypothesis that synaptically released Zn participates in central excitatory neurotransmission is supported by several electrophysiological studies linking alterations in Zn availability to changes in neuronal circuit behavior. Zn application increases the firing of some cortical neurons (*8*) and prolongs the excitatory synaptic potential in olfactory cortical neurons (*9*). In addition, chelation of Zn with diethyldithiocarbamate (*10*) or dietary Zn depletion (*11*) has been reported to produce alterations in mossy fiber transmission.

However, evidence for an appropriate direct membrane action of Zn specific to excitatory synapses has been sparse. Previously described effects of Zn on neuronal membranes, including attenuation of voltage-dependent sodium (*12*) or calcium (*13*) conductances, and potentiation of postsynaptic γ-aminobutyric acid responses (*9*), represent possible avenues of Zn action but lack specificity to excitatory synapses. Most central excitatory synapses, including in particular the hippocampal mossy fiber connections, are likely mediated by the action of glutamate, or related compounds, on perhaps three subclasses of receptors, each defined by a specific agonist: *N*-methyl-D-aspartate (NMDA), quisqualate (Quis), and kainate (Kain) (*14*). We investigated the effect of Zn on the responses to each of these agonists in a cell culture system.

Dissociated mouse cortical cell cultures were prepared and maintained generally as previously described (*15, 16*). The only source of Zn in the culture medium was the serum (Hyclone, defined grade), resulting in an estimated Zn concentration of less than 2 μ*M* in the maintenance medium. Cultures between 15 and 24 days in vitro were mounted on the heated (35°C) stage of an inverted phase-contrast microscope and perfused continuously with a defined, Zn-free recording medium (*p*H 7.3) containing the following (in millimoles per liter): Na, 130; K, 5.4; Ca, 4; Cl, 143; glucose, 15; and Hepes, 10 (salts were from Baker, analyzed reagent grade). Magnesium was omitted from the medium (except as noted below) to facilitate study of responses mediated by the NMDA receptor (*17*); tetrodotoxin (1 μ*M*) was added routinely to reduce interference from spontaneous synaptic activity. Intracellular recordings were made from directly visualized neurons with the use of 4*M* potassium acetate–filled microelectrodes (40 to 100 megohms); current was passed through the recording electrode by means of a standard bridge circuit. Only neurons with stable resting potentials greater than −50 mV and an input time constant permitting unequivocal balancing of the bridge circuit were included in the study.

Pressure ejection (*18*) of 25 to 100 μ*M* NMDA, 100 to 200 μ*M* Kain, or 10 to 50 μ*M* Quis onto cortical neurons reliably produced depolarizing responses associated with increases in membrane conductance (Fig. 1A). Consistent with other data (*19*), this NMDA response could be selectively blocked by 100 to 1000 μ*M* 2-amino-5-phosphonovalerate (*16, 20*). Delivery of 100 μ*M* to 1 m*M* Zn alone (as $ZnCl_2$) generally produced no change in membrane potential or conductance (Fig. 1A), although occasionally (four times in 21 trials in one series), a small 2- to 5-mV transient hyperpolarization was noted (Fig. 3D).

Pressure ejection of 500 μ*M* to 1 m*M* Zn for several seconds always reversibly attenuated (16 of 16 cells) and sometimes completely blocked (8 of 16 cells) the membrane depolarization and conductance increase produced by subsequent application of NMDA (Fig. 1A), resulting in a mean reduction of 85% ± 4% (SEM) in the baseline NMDA depolarization amplitude (Fig. 1B). This attenuation was highly selective for NMDA responses. The same concentrations of Zn reversibly potentiated the response to Quis in most cells tested (17 of 21) (Fig. 1A) while having no effect in a few (4 of 21) cells tested; the mean increase in Quis depolarization amplitude was 26% ± 4% (Fig. 1B). Some potentiation in

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