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Correlation of regional brain metabolism with receptor localization during ketamine anesthesia: Combined autoradiographic 2-[^{3}H]deoxy-D-glucose receptor binding technique

(phencyclidine/opiate receptor/phencyclidine receptor/autoradiography/hippocampus)

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ABSTRACT LKB film autoradiography of 2-[^{3}H]deoxy-D-glucose uptake shows that ketamine, administered in anesthetic doses, alters the pattern of metabolic activity in rat hippocampus. The labeled metabolic marker can be washed out of the slide-mounted tissue sections by preincubation to permit *in vitro* autoradiography of drug and neurotransmitter receptors in the same animal. In this way, opiate and phencyclidine receptor distributions may be correlated with patterns of glucose utilization in adjacent sections. If the observed relative enhancement of 2-deoxy-D-glucose uptake in the stratum moleculare of hippocampus reflects elevated metabolism in nerve terminals there, then the binding of ketamine to phencyclidine receptors on neurons in distant afferent sites, such as entorhinal cortex, may initiate the physiologic and metabolic effects.

Phencyclidine (PCP) analogues, such as ketamine, are frequently used as anesthetics though their locus of action is unknown. Acute administration of PCP in rat alters neurotransmitter levels (1) and metabolism (2) in dopamine-, γ-aminobutyric acid-, and serotonin-containing neuronal systems. PCP has been shown to increase glucose utilization in several limbic cortical regions (3), particularly in the hippocampus (4). Recent pharmacological reports that demonstrate radiolabeled PCP binding to a specific PCP receptor (5–8) reveal that the psychotomimetic "σ opiates" are, in fact, not opiates at all but derive their effect from an ability to interact with the PCP receptor (5, 7, 8). Interactions of PCP derivatives with the opiate receptor have been observed, but very high doses were required to inhibit naloxone binding (9). In this study, we use a double-labeling autoradiographic technique to investigate the correlation between μ-opiate and PCP receptor distributions and the pattern of metabolic changes produced by ketamine.

The method to be described permits simultaneous visualization of the effects of drug administration on brain metabolism and the relevant receptors that mediate the drug's effects. The use of adjacent tissue sections from the same animal to examine regional glucose utilization by the method of 2-deoxy-D-glucose (2dGlc) uptake (10, 11) and receptor binding by *in vitro* autoradiography (12) permits a direct comparison of different variables in the same brain. In alternate sections, the phosphorylated and trapped 2dGlc can be visualized autoradiographically or washed out of the tissues by several rinses prior to incubation in media containing radiolabeled ligands to mark receptor locations. By comparing regional glucose utilization and receptor binding patterns in normal, unanesthetized rats and in rats after acute ketamine administration, the immediate effects of the drug on glucose utilization and on receptor distributions can be examined. From results of this double-labeling procedure, we can suggest that ketamine-altered metabolism in discrete hippocampal regions results from the physiological events that follow its binding to receptors located on entorhinal cortical neurons projecting to these regions.

METHODS

Five female rats weighing 140–150 g were injected with ketamine hydrochloride (100 or 200 mg/kg of body weight, by intravenous infusion) at 0840 hours. After 10–20 min, 1 mCi of [^{3}H]2dGlc (40 Ci/mmol, 1 Ci = 3.7×10^{10} becquerels; New England Nuclear) was injected through the tail vein. Five additional rats received no ketamine anesthesia, and the [^{3}H]2dGlc was injected at 0900. After a 45-min uptake period, during which time the animals remained in a plastic restrainer, animals were decapitated, and brains were removed rapidly, frozen in 2-methylbutane at −35°C, mounted on a pedestal, and sectioned at 25 μm in a −22°C cryostat. For [^{3}H]2dGlc localization, every fifth section was mounted on a cold coverslip which was placed immediately onto a 60°C hot plate and later fixed and processed for autoradiography as described below. For receptor localizations, sets of adjacent sections from two brains were mounted onto cold gelatin-coated glass slides, thaw-mounted, dried at 0°C under reduced pressure, and then stored in a −15°C freezer. Receptor binding was also analyzed in two normal control rats not given 2dGlc prior to decapitation.

Slide-mounted tissue sections for [^{3}H]2dGlc autoradiography (11) were fixed in paraformaldehyde vapors under reduced pressure at 80°C (12) and then apposed to tritium-sensitive LKB Ultrofilm in x-ray cassettes for 24 days before developing. Adjacent sections for receptor localization studies were placed into staining racks and transferred through six 30-sec successive changes of an appropriate preincubation solution to first remove the diffusable [^{3}H]2dGlc. For naloxone binding (12), (*i*) preincubation washing was in 50 mM K_2HPO_4/HCl buffer at pH 7.4 and 0°C, (*ii*) incubation was for 60 min at the same temperature in 300 ml of 50 mM K_2HPO_4 buffer, pH 7.4/100 mM NaCl/1 nM [^{3}H]naloxone (40 Ci/mmol; New England Nuclear), and (*iii*) postincubation washing to remove nonspecifically bound ligand was in six 20-sec rinses at 0°C in phosphate-buffered saline containing 1% bovine serum albumin, after which the slides were dried under a stream of air (12). For PCP binding (5), six washes with 5.0 mM Tris·HCl/50 mM sucrose at pH 7.4 and 0°C to remove the [^{3}H]2dGlc were followed by (*i*) a 15-min preincubation at 0°C in the Tris/sucrose buffer containing 20 mM NaCl, (*ii*) a subsequent single rinse in buffer, (*iii*) a 45-min incubation in 300 ml of the Tris/sucrose buffer containing 8.2 mM [^{3}H]PCP (48 Ci/mmol; New England Nuclear) at 0°C, and

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Abbreviation: PCP, phencyclidine; 2dGlc, 2-deoxy-D-glucose.

then (*iv*) six 30-sec washes in 0°C buffer containing 1% bovine serum albumin and forced air drying of slides. After drying, incubated tissue sections were fixed in paraformaldehyde vapors and exposed to LKB Ultrofilm at room temperature for 3–4 wk. The film was developed in Kodak D-19 for 4 min at 20°C, placed in stop bath for 15 sec, fixed in Kodak Rapid Fixer for 4 min, washed in running tap water, and dried.

To assess the ratio of total to nonspecific binding, some sections were incubated in 30 ml of the incubation solution including nonradioactive 1 μM etorphine or 100 μM PCP for naloxone or PCP binding, respectively. Specific binding for each ligand was similar to that obtained previously with identical

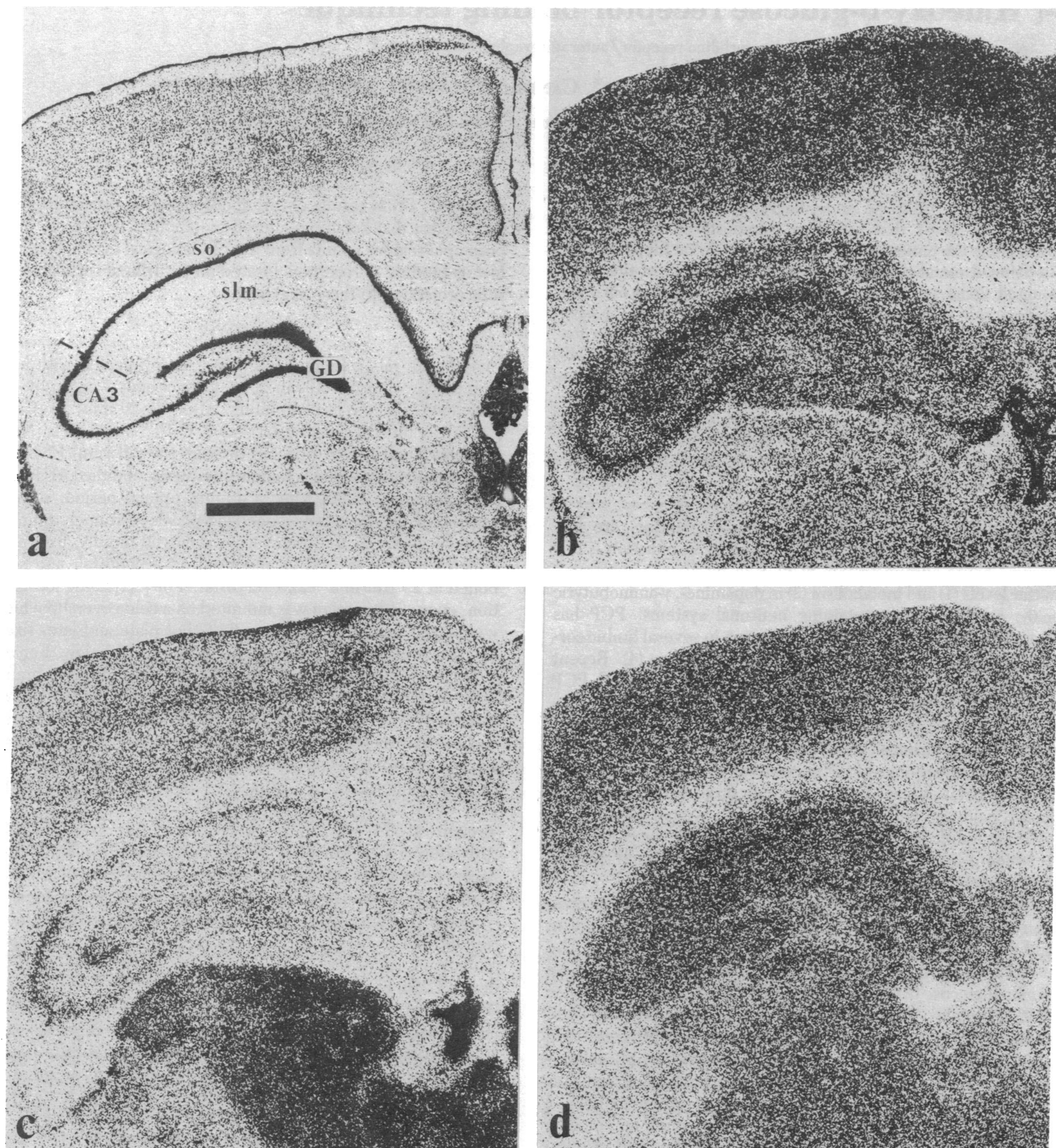

FIG. 1. Comparison of autoradiographic patterns of 2dGlc uptake, opiate receptors, and PCP receptors in the hippocampus of a ketamine-injected rat. (*a*) Thionin-stained section after processing for [^{3}H]naloxone binding (its autoradiograph is shown in *c*). The histologic quality of the tissue is not affected by the double-labeling procedure. so, Stratum oriens; slm, stratum lacunosum–moleculare; GD, dentate gyrus; CA3, cornu Ammonis area 3 [after Lorente de No (13)] demarcated from the adjacent region by a dashed line. Pyramidal and granule cell layers of hippocampus and dentate gyrus, respectively, are darkly stained. The choroid plexus shows medially in the subarachnoid space and laterally in the lateral ventricle. (*b–d*) LKB Ultrofilm autoradiographs of three adjacent sections from the same animal. (*b*) [^{3}H]2dGlc utilization pattern after ketamine injection. (*c*) [^{3}H]Naloxone binding to opiate receptors. (*d*) [^{3}H]PCP binding to PCP receptors. (Bar = 1 mm.)

binding conditions (5, 12). The adequacy of [^{3}H]2dGlc removal in the preincubation washes was quantified after the initial wash by counting a tissue-laden slide fragment in 10 ml of Aquassure scintillation cocktail (New England Nuclear). Counts were reduced from 1280 dpm per section to background levels.

RESULTS AND DISCUSSION

The effects of ketamine on metabolism are evident by comparing the [^{3}H]2dGlc patterns in unanesthetized, restrained rats (Fig. 2*b*) with those in ketamine-anesthetized rats (Fig. 1*b*). The appearance of laminated metabolic activity in the hippocampus of control animals reflects the enhanced resolution achieved by the use of ^{3}H rather than ^{14}C label. In the ketamine-anesthetized animal (Fig. 1*b*), the [^{3}H]2dGlc uptake in the hippocampus is characterized by a dense band of grains overlying the stratum lacunosum–moleculare of Ammon's horn and the molecular layer of the dentate gyrus, in accord with Lund *et al.* (4). Densitometry (13) has shown that the ratio of 2dGlc uptake in gray matter of the superficial hippocampal layers to white matter of the corpus callosum is increased by 35–45% over the same ratio in the unanesthetized control rat. Moreover, there is an overall increase of 2dGlc uptake in the hippocampus relative to the thalamus and adjacent cortex in the ketamine-treated animal. Quantitative data from previous studies suggest that this increase results from a 20% increase in metabolism in the hippocampus (3), which in large part must be due to the selective increase of 2dGlc uptake by the stratum lacunosum–moleculare.

Opiate receptors in both ketamine-injected and uninjected animals were indistinguishable and in accord with previous works (14–16); hippocampal [^{3}H]naloxone binding was dense in a band within the stratum oriens and the pyramidal cell layer and in another band in the stratum lacunosum–moleculare, especially in CA3; the binding was somewhat less dense in the hilus of the dentate gyrus (Fig. 1*c*). This pattern, from tissue which had been washed to remove the metabolized [^{3}H]2dGlc, appears to be identical to that from an animal which received no prior ketamine or 2dGlc injection or preincubation wash (Fig. 2*a*), indicating that neither the extra washing required for the double-labeling procedure nor prior drug injection had any effect on opiate binding or tissue quality (Fig. 1*a*).

Likewise, the pattern of PCP receptor distribution shown here (Fig. 1*d*) is indistinguishable from that previously described (5). PCP receptor labeling in the hippocampus showed a slight laminar pattern characterized by heavier labeling of PCP receptors in superficial layers and less labeling in the granule cell layer of the dentate gyrus and along the hippocampal fissure (Fig. 1*d*). In addition, the hippocampus had more [^{3}H]PCP binding than had the adjacent cortex or thalamus.

These results show that although acute administration of ketamine profoundly alters the pattern of regional brain metabolism, it does not affect the localization of opiate or PCP receptors, which presumably represent the initial sites of action of the drug. The altered [^{3}H]2dGlc pattern reflects the physiological consequence of drug–receptor interactions; the receptors themselves are relatively stable membrane-bound proteins unaffected by acute treatment. The similarity of receptor binding patterns in ketamine-treated and control tissue indicates that prior ketamine administration does not block binding of the [^{3}H]PCP ligand to its receptor. Perhaps the preincubation used to remove 2dGlc from the preparation also serves to eliminate ketamine bound to the receptor.

Comparison of the [^{3}H]2dGlc-labeling pattern with receptor binding in hippocampal laminae by means of the double-label-

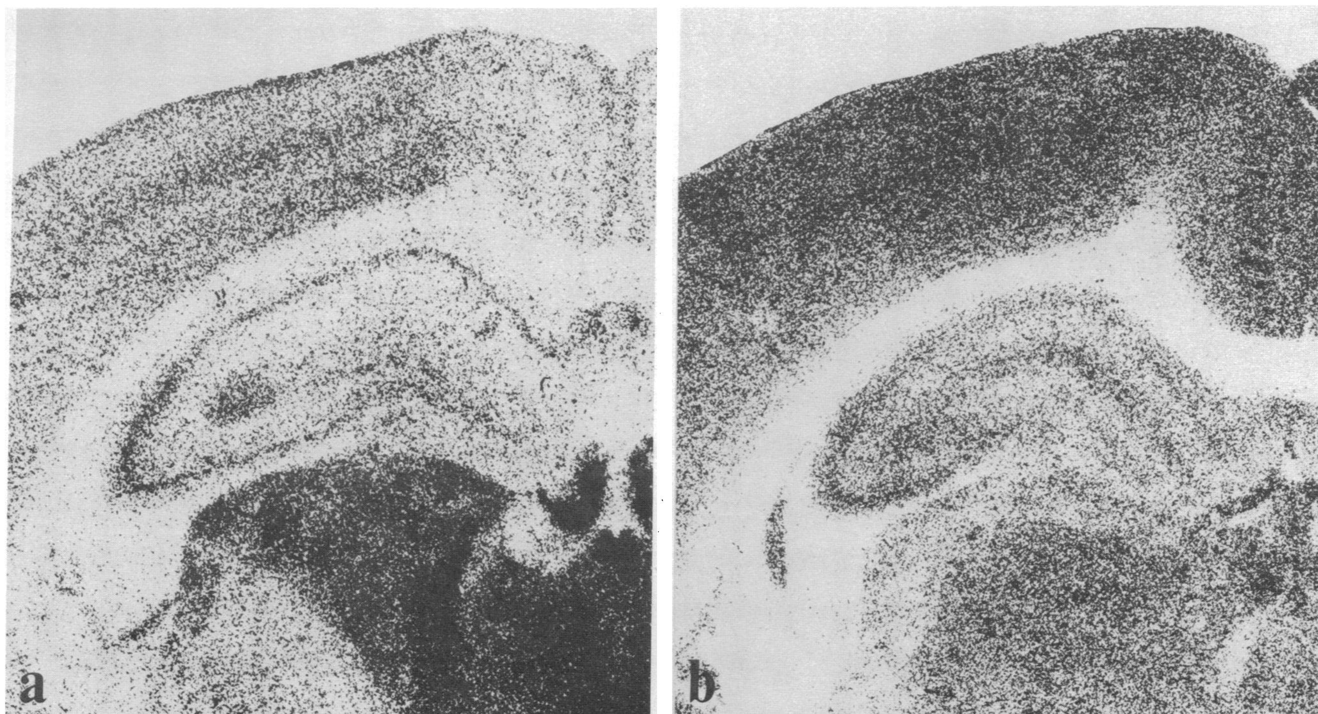

FIG. 2. LKB film autoradiographs of sections from control animals not given ketamine or 2dGlc before sacrifice. (*a*) [^{3}H]Naloxone binding to opiate receptor as described (12). (*b*) [^{3}H]2dGlc uptake in tissue from an awake, restrained, and unanesthetized animal. Resolution is adequate to reveal differential 2dGlc uptake in the cerebral cortex and the hippocampus. Relatively high levels of 2dGlc uptake occur in the cortex in layer 4, in the thalamus throughout most nuclei, and in the hippocampus in the stratum oriens, particularly in the cornu Ammonis area 3, the stratum lacunosum–moleculare, and the molecular layer of the dentate gyrus. Uptake is low in pyramidal and granule cell layers of hippocampus and dentate gyrus, respectively.

ing technique may elucidate the anatomical circuitry by which ketamine selectively alters metabolism. For instance, the pyramidal cell layer and adjacent stratum oriens were heavily labeled by both [^{3}H]2dGlc and [^{3}H]naloxone, as was the molecular layer of CA3 (Figs. 1*b* and *c*). Opiate receptors were present also, though in lesser number, in the superficial layer of CA1, where the ketamine effect on metabolism appeared most profoundly. PCP receptors, by contrast, were much more uniformly distributed. Patterns of receptor location need not be in register with the sites of metabolic consequence of drug–receptor interactions because receptors are often located on the postsynaptic membranes, whereas metabolic effects may be greatest in the presynaptic elements (17). Ketamine-induced alterations in 2dGlc uptake may result from drug activation of distantly located neurons that send their axons to terminate in the region of altered metabolism. The increased 2dGlc uptake in the hippocampal molecular layer may reflect greater utilization in terminals of entorhinal perforant path axons, which make synaptic contact on the apical dendrites of hippocampal pyramidal neurons at precisely this locus (18, 19). In fact, it has been shown that electrical stimulation of the perforant path from entorhinal cortex yields a distinct band of increased 2dGlc uptake in the molecular layer of hippocampus and dentate gyrus (20) similar to that observed during ketamine exposure. Because the entorhinal area contains high densities of PCP receptors (5), ketamine binding to the PCP receptors in this region may underlie the hippocampal pattern of increased [^{3}H]2dGlc metabolism. The use of high-resolution, tritium-labeled metabolic and receptor markers in adjacent sections from the same animal can be used as a general method for correlating the location of neurohumoral receptors with the metabolic consequences of their activation by pharmacological or endogenous substances.

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