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Autoradiographic Analysis of the In Vivo Distribution of ^{3}H-Imipramine and ^{3}H-Desipramine in Brain: Comparison to In Vitro Binding Patterns[1]

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DUNCAN, G. E., I. A. PAUL, J. B. FASSBERG, K. R. POWELL, W. E. STUMPF AND G. R. BREESE. *Autoradiographic analysis of the in vivo distribution of ^{3}H-imipramine and ^{3}H-desipramine in brain: Comparison to in vitro binding patterns.* PHARMACOL BIOCHEM BEHAV **38**(3) 621–631, 1991.—Using high resolution autoradiographic techniques, the distribution of radioactivity in forebrain and brainstem was assessed after IV injection of ^{3}H-impramine or ^{3}H-desipramine. Results were compared with regional binding of the drugs to brain sections in vitro. Similar topographic binding of ^{3}H-imipramine and ^{3}H-desipramine was observed in vitro among brain regions, except in the paraventricular nucleus of the hypothalamus and locus coeruleus, where binding was greater for ^{3}H-desipramine. For both ^{3}H-desipramine and ^{3}H-imipramine, some brain regions that exhibited high binding in vitro also showed high accumulation after in vivo injection. However, certain regions that contained high densities of binding sites for the antidepressant drugs as measured by in vitro binding showed very low accumulation of radioactivity after in vivo treatment. Such regions included the dentate gyrus of the hippocampus, layer 1 of piriform cortex, caudate-putamen, pontine and midbrain central gray, and cerebellar granular layer. Compared to in vitro binding of the drugs, the distribution of imipramine and desipramine in vivo appears more anatomically selective. For imipramine, primary sites of action in vivo, as indicated by the topographic distribution in brain, appear to be the locus coeruleus, hippocampus, lateral septal nucleus, and amygdala. For desipramine, the greatest accumulation in vivo was found in the locus coeruleus, paraventricular nucleus of the hypothalamus, and anterior thalamic nuclei.

Antidepressants Autoradiography In vivo binding In vitro binding Imipramine Desipramine
Locus coeruleus Hippocampus

IMIPRAMINE was one of the first drugs found to be effective in the treatment of depression and remains a widely used antidepressant drug. An active metabolite of imipramine, desipramine, is also an effective antidepressant. Imipramine and desipramine are potent inhibitors of norepinephrine and serotonin uptake in synaptosomal fractions (21,26). Receptor-like binding sites for ^{3}H-imipramine and ^{3}H-desipramine in brain have been demonstrated in vitro by biochemical (15, 18, 19) and autoradiographic techniques (4, 5, 8, 11, 13). Both approaches have shown that recognition sites for the drugs are heterogeneously distributed in brain.

Although apparently not recognized previously, available anatomical data for the in vivo distribution of imipramine in brain (5,22) suggest that there may be distinct anatomical differences in the localization of imipramine after systemic injection, compared to the reported topography of ^{3}H-imipramine binding to brain sections in vitro (4, 8, 11, 13). The present study provides a detailed autoradiographic analysis of the distribution of ^{3}H-imipramine and ^{3}H-desipramine in the forebrain and brainstem in vivo and compares the in vivo drug distribution with topographic patterns of drug binding to brain sections in vitro.

METHOD

In Vivo Distribution of ^{3}H-Imipramine and ^{3}H-Desipramine

For in vivo studies, rats (male Sprague-Dawley, Charles River Inc., Raleigh) were implanted with jugular catheters that were exteriorized at the dorsal aspect of the neck (10) to permit IV in-

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jection of ^{3}H-imipramine and ^{3}H-desipramine under conditions of minimal stress. Animals recovered at least 48 h after surgery before the radiolabelled compounds were administered. Radiolabeled drugs (^{3}H-imipramine, 74 Ci/mmole, ^{3}H-desipramine, 63 Ci/mmole) were purchased from New England Nuclear (Boston, MA) and injected via the catheter. Rats were killed by decapitation and brains processed for autoradiography or dissected for measurement of radioactivity by liquid scintillation counting.

In studies to define the time course of ^{3}H-imipramine in brain after IV injection and to determine if unlabeled imipramine could displace ^{3}H-imipramine binding in vivo, experiments were conducted with dissected brain regions. For these studies, rats (Sprague-Dawley, Charles River Inc., Raleigh) were injected with ^{3}H-imipramine (0.05 to 0.2 μCi/g body weight) and killed 5 to 60 min after injection. Frontal cortex, hippocampus, and cerebellum were solubilized in 1.0 ml of NCS medium (Amersham). Samples were neutralized with acetic acid and 10 ml of Scintiverse II (Fisher) added for quantification of radioactivity by liquid scintillation counting.

For in vivo autoradiographic studies, rats were killed 5 or 60 min after IV injection of ^{3}H-imipramine (1.6 μCi/body weight, n=4) or 60 min after ^{3}H-desipramine (1.6 μCi/g body weight, n=4). Brains were frozen in liquid propane (−180°C) cooled with liquid nitrogen. Autoradiograms were produced as described in detail (10, 12, 24). Four μm cryostat sections were thaw-mounted onto Kodak NTB-3 nuclear emulsion coated slides and autoradiograms were exposed 1 to 10 months in light-tight desiccator boxes before photographic processing. The relative distribution of radioactivity among brain regions was quantitated by digital image analysis using a charge couple device camera and custom designed software. Results are expressed as optical density ratios of brain regions relative to that of the lateral habenula. For each brain region, three sections were analyzed for each rat.

In Vitro Binding of ^{3}H-Imipramine and ^{3}H-Desipramine

For in vitro binding studies, 10 μm brain sections were thaw-mounted onto cover slips and incubated at 0°C for 60 min with either 5 nM ^{3}H-imipramine or 5 nM ^{3}H-desmethylimipramine in 50 mM trihydroxyaminomethane-HCl (Tris, pH 7.4) containing 150 mM NaCl. Nonspecific binding was assessed in the presence of 100 μM imipramine and accounted for 30–50% of the total binding. Following incubation with radiolabeled compounds, sections were washed by immersion into 2 changes of ice-cold Tris buffer for 5 min each, followed by a 3-min immersion into distilled water to remove buffer salts. Sections were dried in a stream of cool air and apposed to Kodak NTB-3 emulsion coated slides in an X-ray cassette. Optical densities in autoradiograms were analyzed as described above.

Statistics

Comparisons of the in vivo distribution and in vitro binding data were made by repeated measures analysis of variance. Results were deemed significantly different when $p<0.05$.

RESULTS

Time Course of Radioactivity in Brain After IV Injection of ^{3}H-Imipramine

Rats were injected with ^{3}H-imipramine IV and sacrificed 5 to 60 min later. Radioactivity in brain regions was high 5 min after administration of ^{3}H-imipramine and declined rapidly, such that the amount of radioactivity present at 60 min was approximately ⅓ of that found at 5 min (Fig. 1).

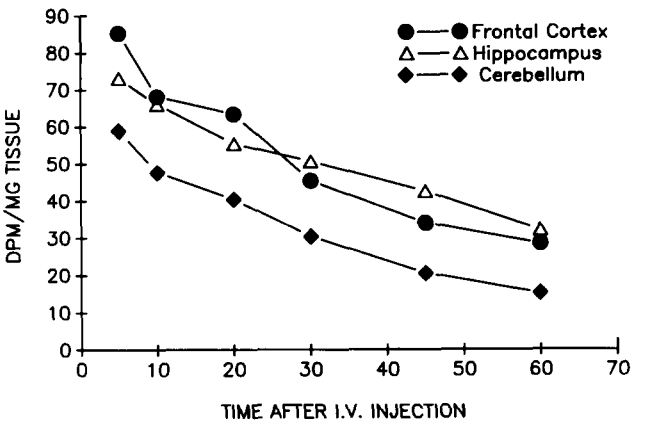

FIG. 1. Time course of radioactivity in cerebellum, hippocampus, and frontal cortex after IV injection of ^{3}H-imipramine. Rats were killed at the indicated times and dissected brain regions were solubilized for determination of radioactivity by liquid scintillation counting. The hippocampus includes Ammon's horn and the dentate gyrus. Autoradiographic results below show that Ammon's horn contains high levels of radioactivity, whereas the dentate gyrus contains low amounts of radioactivity, sixty min after injection.

In Vitro Binding of ^{3}H-Imipramine to Brain Sections

A distinct topographic distribution of binding was apparent after incubation of brain sections in vitro with ^{3}H-imipramine. In vitro binding of ^{3}H-imipramine was high in the glomerular and external plexiform layers of the olfactory bulb, layer 2 of the piriform cortex, isocortical regions, lateral septal nucleus, anterior thalamic nuclei, stratum oriens, and stratum radiatum of the hippocampus, molecular layer of the dentate gyrus, gray matter areas of the caudate putamen. The cerebellar granule cell layer also displayed high binding of ^{3}H-imipramine. In the brain stem the pontine and midbrain central gray and the dorsal raphe were found to contain high densities of binding sites for the drug. Results of quantitative analysis of optical densities are provided in Table 1 and photomicrographs of representative autoradiograms are presented in Figs. 2–6.

Comparison of In Vitro Binding With In Vivo Distribution of ^{3}H-Imipramine

The distribution of radioactivity in brain after IV injection of ^{3}H-imipramine was distinctly different from the topography of ^{3}H-imipramine binding to sections in vitro. Many brain regions that exhibited very low levels of radioactivity 60 min after IV injection of ^{3}H-imipramine contained high densities of binding sites for the drug in vitro (Table 1, Figs. 2–6). Especially notable are the differences in the dentate gyrus for the in vivo distribution compared to in vitro binding (Figs. 4, 5, and 8). After in vivo treatment with ^{3}H-imipramine, the molecular layer of the dentate gyrus contained the lowest amount of radioactivity in the forebrain but this region exhibited high ^{3}H-imipramine binding in vitro. Other regions that showed greater relative binding in vitro compared to in vivo include the cerebellar granule cell layer (Fig. 7), pontine (Fig. 7) and midbrain (Fig. 6) central gray, gray matter regions of the caudate putamen (Figs. 3–5), layer 2 of the piriform cortex (Fig. 3), and external plexiform layer of the ol-

TABLE 1

QUANTITATIVE EVALUATION OF THE RELATIVE IN VIVO DISTRIBUTION AND IN VITRO BINDING OF ^{3}H-IMIPRAMINE

Brain Region	Ratio of Silver Grain Density to Lateral Habenula	
	In Vivo	In Vitro
CA-1, Stratum Radiatum	1.76 ± 0.16	1.70 ± 0.09
CA-1, Stratum Oriens	1.80 ± 0.12	1.74 ± 0.12
Dentate Molecular	1.07 ± 0.05	2.11 ± 0.23*
Cingulate Cortex, Layer 1	1.29 ± 0.06	1.57 ± 0.09
Caudate-Putamen	1.13 ± 0.06	1.56 ± 0.14*
Piriform Cortex, Layer 1	0.99 ± 0.07	2.07 ± 0.15*
Lateral Septal Nucleus	1.49 ± 0.06	1.46 ± 0.07
Basolateral Amygdala	1.53 ± 0.05	1.52 ± 0.08
Dorsal Raphe	1.48 ± 0.07	1.77 ± 0.10*
Midbrain Central Gray	1.16 ± 0.06	1.61 ± 0.09*
Locus Coeruleus	2.56 ± 0.16	1.44 ± 0.07*

For the in vivo condition, rats were sacrificed 60 min after IV injection of ^{3}H-imipramine (1.6 μCi/g body weight). For the in vitro condition, sections were incubated in 5 nM ^{3}H-imipramine. Data are mean ± S.D.
*$p<0.05$ compared to in vivo binding.

factory bulb (Fig. 2). However, relatively high binding of ^{3}H-imipramine occurred both in vivo and in vitro in the stratum oriens and stratum radiatum of the hippocampus, dorsal lateral septal nucleus, basolateral nucleus of the amygdala, and dorsal raphe nucleus.

The locus coeruleus was densely labeled after in vivo administration of ^{3}H-imipramine, in contrast to the intermediate binding of the compound in vitro. Dense foci were labeled in the accessory olfactory nucleus after in vivo treatment with ^{3}H-imipramine, whereas this region displayed diffuse and relatively low binding of the drug in vitro. The lateral habenular nucleus contained very low levels of radioactivity in both the in vivo and in vitro conditions.

Autoradiographic Evaluation of the Distribution of ^{3}H-Imipramine in Brain 5 Min After IV Injection

Low binding of ^{3}H-imipramine in vivo to certain brain regions, relative to in vitro, could be due to low delivery of ^{3}H-imipramine to certain regions in vivo. To test that possibility, rats were killed 5 min after IV injection of ^{3}H-imipramine and their brains processed for autoradiography. As found in the studies with solubilized tissue, the amount of radioactivity in brain regions examined was much greater at 5 min compared to 60 min after IV injection of ^{3}H-imipramine. Exposure periods of 2 months for the 5-min survival produced comparable optical densities to 8 months exposure periods for the 60-min survival. The topographic distribution of ^{3}H-imipramine 5 min after IV injection was distinctly different from that observed 60 min after injection (Fig. 8, and compare Figs. 3d with 9a and 5d with 9b). At five minutes, the distribution of ^{3}H-imipramine in brain is similar to regional blood flow, with the highest amounts of radioactivity present in the cerebral cortex. The molecular layer of the dentate gyrus and the caudate-putamen also contained relatively high levels of radioactivity 5 min after injection of ^{3}H-imipramine, in contrast to the very low levels of radioactivity in these regions 60 min after-injection. At 5 min after injection, the stratum radiatum of the hippocampus contained relatively low levels of radioactivity, whereas this region contained the highest amount of radioactivity in the forebrain at 60 min. Thus low delivery of ^{3}H-imipramine to certain regions in vivo cannot explain the differences between in vivo and in vitro binding of the compound.

Comparison of In Vitro Binding With the In Vivo Distribution of ^{3}H-Desipramine: Relationship to ^{3}H-Imipramine

Since imipramine is metabolized to desipramine in vivo, the in vivo distribution and in vitro binding of ^{3}H-desipramine was examined. The neuroanatomical distribution of ^{3}H-desipramine binding in vitro was similar to that of ^{3}H-imipramine binding in

TABLE 2

EFFECT OF PRETREATMENT WITH UNLABELED IMIPRAMINE ON IN VIVO ^{3}H-IMIPRAMINE ACCUMULATION[a]

Brain Region	Saline Pretreatment	Imipramine Pretreatment (20 mg/kg)	Saline Pretreatment	Imipramine Pretreatment (2 × 20 mg/kg)
Cerebellum	16.3 ± 0.4	18.7 ± 1.6	75.5 ± 6.0	124.5 ± 7.7*
Frontal Cortex	31.4 ± 2.4	29.5 ± 2.9	127.8 ± 12.0	188.0 ± 12.7*
Hippocampus (Ammon's horn + dentate gyrus)	36.7 ± 3.2	39.8 ± 1.6	111.8 ± 10.5	185.3 ± 11.2*

[a]For the one dose unlabeled imipramine treatment, rats (5/group) were given IP injections of saline or imipramine (20 mg/kg) one hour before IV injection of ^{3}H-imipramine (0.05 μCi/g body weight). For the two dose schedules, rats (5/group) were injected with saline or unlabeled imipramine 60 min and 15 min before IV injection of ^{3}H-imipramine (0.2 μCi/g body weight). All rats were killed 1 h after the IV injection and radioactivity in dissected brain regions assessed by liquid scintillation counting. Data are dpm/g wet weight tissue, mean ± S.D.
*$p<0.05$ compared to saline pretreatment.

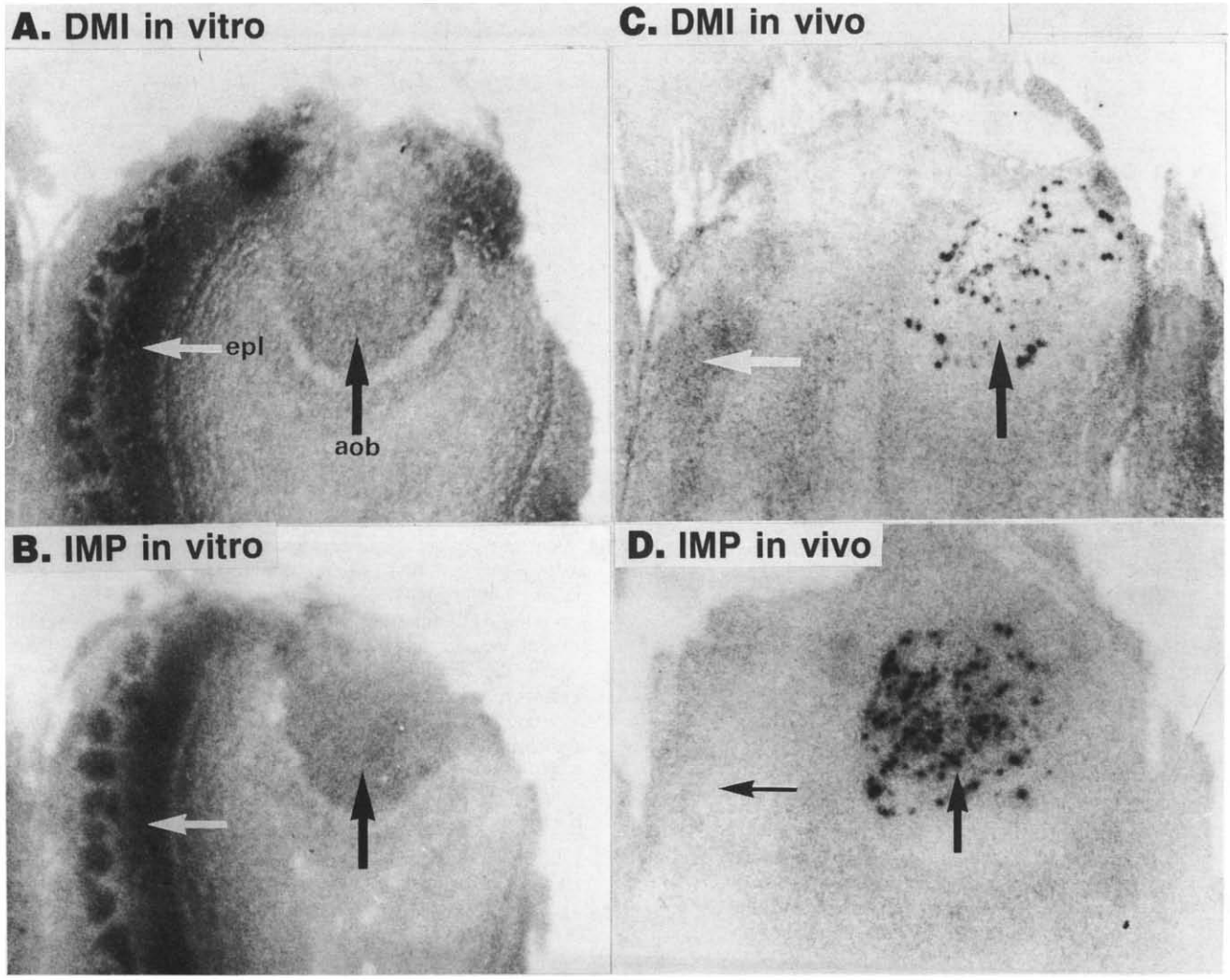

FIG. 2. In vitro binding and in vivo distribution of ^{3}H-desipramine and ^{3}H-imipramine in the olfactory bulb. Abbreviations: epl, external plexiform layer; aob, accessory olfactory bulb. For the in vivo conditions, rats were killed 60 min after IV injection of radiolabeled drugs. In vitro binding was assessed by incubation of brain sections with 5 nM ^{3}H-imipramine or ^{3}H-desipramine. In Figs. 2–7 autoradiographic exposure times were as follows: desipramine (DMI) in vitro 1 month; DMI in vivo 8 months; imipramine (IMP) in vitro 2 months; IMP in vivo 8 months.

vitro (Figs. 2–7), except in the paraventricular nucleus of the hypothalamus (Fig. 4) and the locus coeruelus (Fig. 7), where greater relative binding was present for ^{3}H-desipramine.

As with ^{3}H-imipramine, certain regions that exhibited high binding of ^{3}H-desipramine in vitro contained low amounts of radioactivity after in vivo administration (Figs. 2–7). Such regions include the caudate-putamen (Fig. 3), molecular layer of the dentate gyrus (Figs. 4 and 5), midbrain (Fig. 6) and pontine (Fig. 7) central gray, and cerebellar granule cell layer (Fig. 7). The locus coeruleus (Fig. 7) and paraventricular nucleus (Fig. 4) contained high amounts of radioactivity after injection of ^{3}H-desipramine.

Differences and similarities were apparent for the in vivo distribution of radioactivity after IV injection of ^{3}H-desipramine compared to ^{3}H-imipramine (Figs. 2–7). The locus coeruleus appeared similarly labeled after in vivo treatment with the two antidepressants. Greater accumulation of radioactivity in vivo was apparent for ^{3}H-desipramine in the anterior thalamic nuclei, and paraventricular nucleus of the hypothalamus (Fig. 4). Conversely, greater accumulation of radioactivity in vivo was apparent for ^{3}H-imipramine in the stratum radiatum and stratum oriens of the hippocampus (Figs. 4 and 5).

Attempt to Displace ^{3}H-Imipramine Binding In Vivo With Unlabeled Imipramine

An experiment was conducted to determine if the in vivo binding of ^{3}H-imipramine in brain could be displaced by unlabeled imipramine. Rats were given IP injections of unlabeled imipramine (20 mg/kg) 60 min before IV administration of ^{3}H-imipramine and sacrificed 60 min after the IV injection. Brain regions were dissected and solubilized for assessment of radioactivity by liquid scintillation counting. Pretreatment with unlabeled imipramine did not reduce the amount of radioactivity in brain regions (Table 2). A higher dose of imipramine was also used in an at-

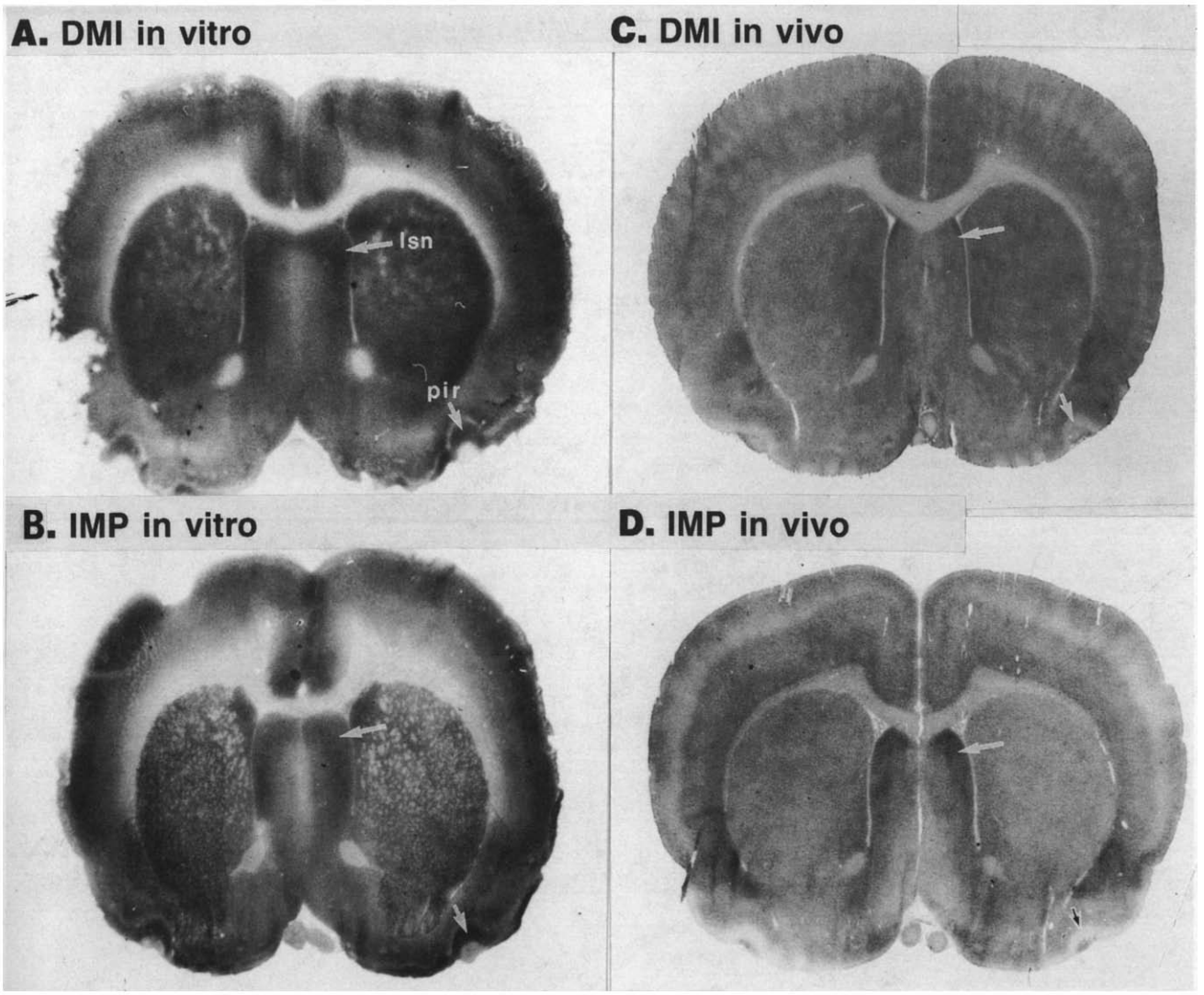

FIG. 3. In vitro binding and in vivo distribution of ^{3}H-desipramine and ^{3}H-imipramine at the level of the medial and lateral septal nuclei. Abbreviations: lsn, lateral septal nucleus; pir, layer 1 of the piriform cortex.

tempt to displace ^{3}H-imipramine binding in vivo. Two IP injections of imipramine were administered 60 min and 15 min (20 mg/kg each) before IV injection of ^{3}H-imipramine. Paradoxically, pretreatment with imipramine using the two dose schedule resulted in greater accumulation of radioactivity in all brain regions examined, compared to saline-treated controls (Table 2).

DISCUSSION

For some psychotropic drugs, the relative neuroanatomical binding to brain sections in vitro is similar to that observed in brain after systemic administration (14,15). However, from the limited neuroanatomical information provided by Cassano and Hannson (6) regarding the distribution of ^{14}C-imipramine in the mouse brain after systemic injection of the compound, it appeared that the relative binding of imipramine in vivo was different from that described in recent in vitro autoradiographic studies (4, 8, 11, 13). The present work provides the most complete anatomical description of the in vivo distribution of ^{3}H-imipramine in brain to date and confirms results of previous in vivo distribution studies with imipramine (6,23) that found high amounts of the drug in the hippocampus relative to other forebrain regions after in vivo treatment. In addition, the present study provides the first direct comparison of the neuroanatomical topography of ^{3}H-imipramine binding in vitro with the distribution of ^{3}H-imipramine in vivo. Comparison of the in vivo distribution and in vitro binding patterns of ^{3}H-imipramine demonstrates that dramatic differences exist between the two conditions for certain brain regions. For example, the molecular layer of the dentate gyrus, layer 2 of the piriform cortex, and caudate-putamen show high binding of imipramine in vitro but very low amounts of radioactivity after in vivo treatment. However, for other brain regions, such as in the stratum oriens and stratum radiatum of the hippocampus, lateral septal nucleus, and basolateral nucleus of the amygdala, the in vivo distribution and in vitro binding of ^{3}H-imipramine was similar.

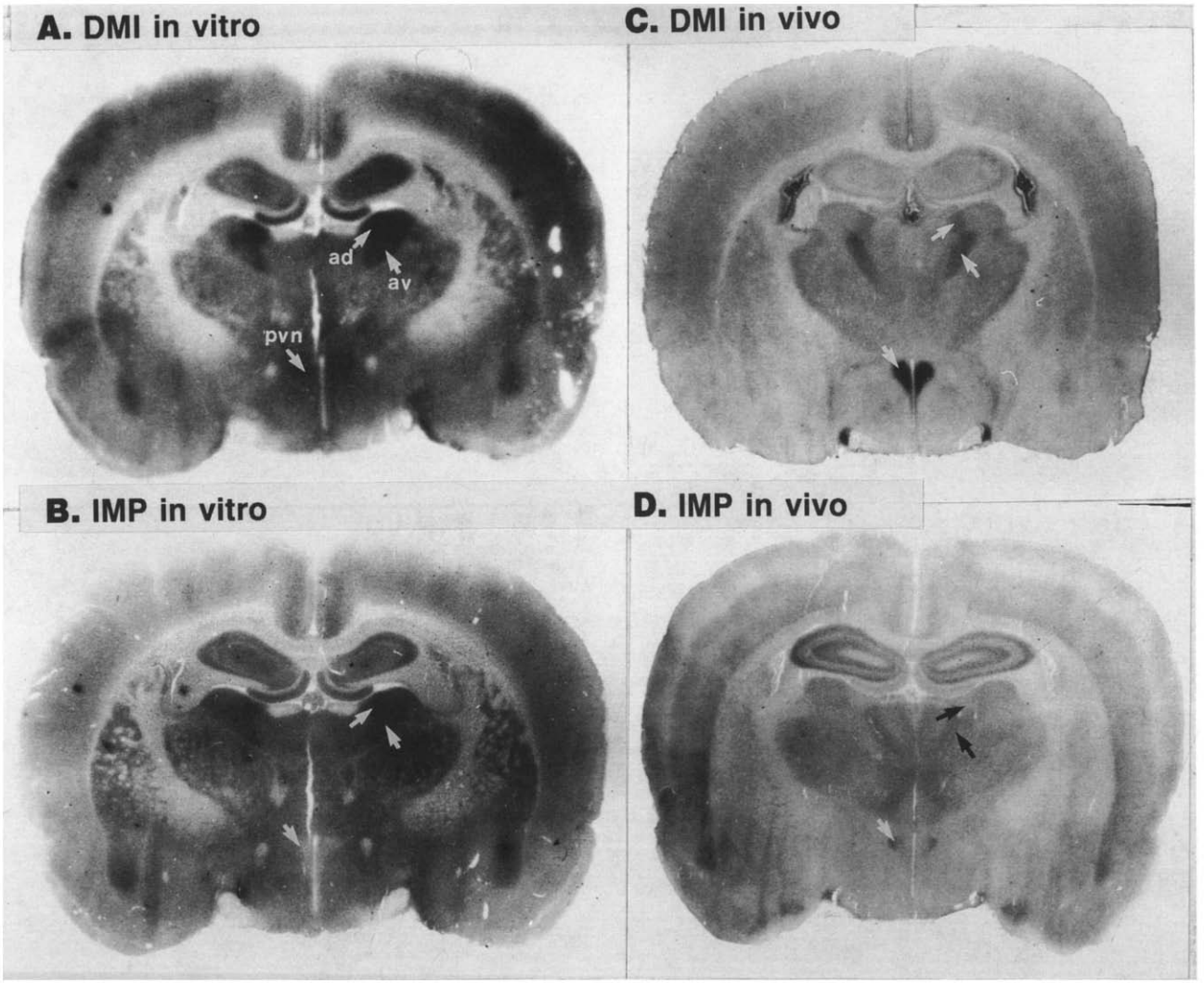

FIG. 4. In vitro binding and in vivo distribution of ^{3}H-desipramine and ^{3}H-imipramine at the level of the anterior hypothalamus. Abbreviations: ad, anterior dorsal thalamic nucleus; av, anterior ventral thalamic nucleus; pvn, paraventricular nucleus (magnocellular part). Note that for desipramine in vivo, high radioactivity is present in the magnocellular (lateral aspect) and parvocellular (medial aspect) of the paraventricular nucleus; in contrast to imipramine in vivo, where only the magnocellular component of the paraventricular nucleus is labeled.

Regional variations in blood flow can be a determinant of the distribution of a drug in brain after systemic administration. Therefore, the possibility was examined that low delivery of ^{3}H-imipramine after IV injection accounts for the relatively low amounts of radioactivity observed in certain brain regions in vivo compared to in vitro. When rats were killed 5 min after IV injection of ^{3}H-imipramine, the amount of radioactivity in brain regions examined was much greater compared to the 60-min survival period. The relative distribution of radioactivity was also distinctly different for the 5-min survival period compared to the 60-min survival time. Five min after injection of ^{3}H-imipramine, the distribution of radioactivity was similar to previously described regional blood flow (22), with cortical regions showing the highest amount of radioactivity. Two regions that contained low levels of radioactivity 60 min after injection of ^{3}H-imipramine, the caudate putamen and the molecular layer of the dentate gyrus, contained relatively high amounts of radioactivity 5 min after injection of the drug. In contrast, relatively low amounts of ^{3}H-imipramine were present 5 min after injection in the stratum radiatum of the hippocampus, whereas this region contained the highest level of radioactivity in the forebrain after a 60-min survival period. Thus it can be concluded that low delivery of ^{3}H-imipramine in vivo does not explain the dramatic differences observed between the in vivo distribution and in vitro binding of the drug at specific brain sites. The greater accumulation of radioactivity in brain 5 min compared to 60 min after IV injection of ^{3}H-imipramine, and the distinctly different distribution of radioactivity at the two time periods after treatment, suggest that an important factor that determines the topographic distribution of ^{3}H-imipramine in brain 60 min after IV injection is differential rate of loss of ^{3}H-imipramine from specific brain regions. Thus regions from which the rate of loss is slower, due to association of the compound with "receptors" or other mechanisms of sequestration, exhibit the greatest amount of radioactivity 60 min after an IV pulse.

Imipramine is metabolized to desipramine in vivo (2). It was possible that the discrepancies between in vitro and in vivo binding of ^{3}H-imipramine were due to metabolism of ^{3}H-imipramine to ^{3}H-desipramine in vivo. Therefore, we assessed ^{3}H-desipramine

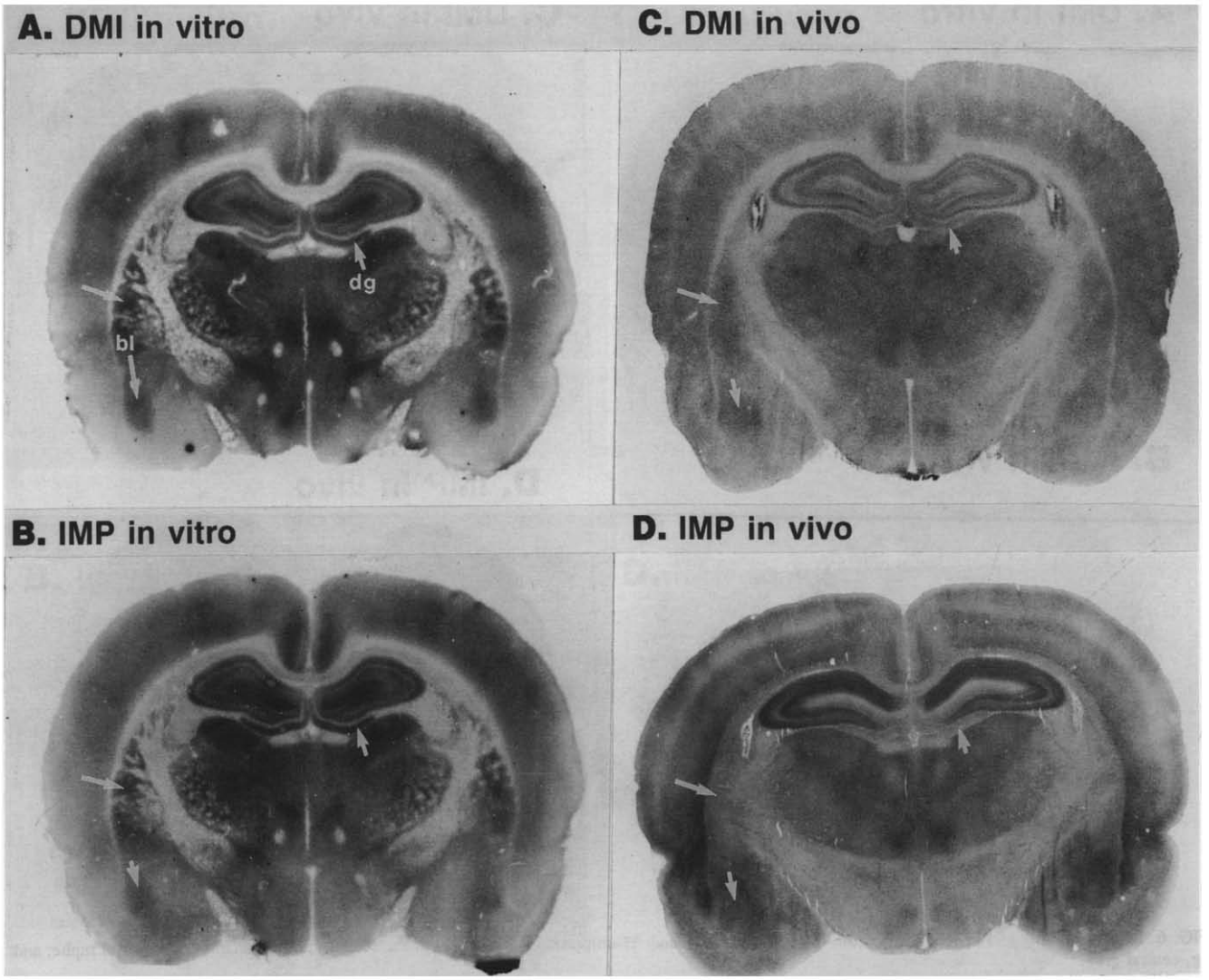

FIG. 5. In vitro binding and in vivo distribution of ^{3}H-desipramine and ^{3}H-imipramine at the level of the central hypothalamus. Abbreviations: dg, dentate gyrus molecular layer; bl, basolateral nucleus of the amygdala.

binding in vitro and the distribution of the drug in brain after in vivo treatment. It was reasoned that if the pattern of distribution of radioactivity in brain after IV injection of ^{3}H-imipramine was due to ^{3}H-desipramine binding, then the in vitro binding of ^{3}H-desipramine should appear similar to results observed for ^{3}H-imipramine in vivo. However, the relative binding of ^{3}H-desipramine in vitro among most brain regions examined was not different from ^{3}H-imipramine, except in the locus coeruleus and paraventricular nucleus, where binding was greater for ^{3}H-desipramine. Thus most differences in the neuroanatomical pattern of distribution of radioactivity observed after in vitro incubation of brain sections with ^{3}H-imipramine, compared to in vivo administration of the compound, can not be explained by metabolism of ^{3}H-imipramine to ^{3}H-desipramine.

It is well documented in previous work (4,5) and in the present study that substantial differences exist in the regional binding of ^{3}H-imipramine and ^{3}H-desipramine in certain brain regions, such as the locus coeruleus and paraventricular nucleus. Although the differences in binding of the two drugs has been emphasized (4,5), close examination of the autoradiograms in those studies reveal that the topography of binding is similar in many regions. In a direct comparison of ^{3}H-imipramine and ^{3}H-desipramine binding in vitro, we demonstrated virtually identical labeling patterns in the cortex, amygdala, hippocampus, and thalamus for the two compounds. The similarities in binding for ^{3}H-desipramine and ^{3}H-imipramine in many brain regions is consistent with the similar pharmacology of the drugs. Although not widely appreciated, imipramine, like desipramine, is a more potent inhibitor of norepinephrine uptake than of serotonin uptake in synaptosomal preparations (21,26).

In the brain stem, the distribution of radioactivity after systemic injection of ^{3}H-imipramine and ^{3}H-desipramine was similar, with high concentrations of radioactivity present in the locus coeruleus. It is possible that the high amounts of radioactivity present in the locus coeruleus after injection of ^{3}H-imipramine represent, in part, the metabolite ^{3}H-desipramine. In forebrain regions, 60 min after IV injection of ^{3}H-imipramine and ^{3}H-desipramine, distinct differences were observed for the distribution of radioactivity in brain. The stratum radiatum of the hippocampus was densely labeled after injection of ^{3}H-imipramine but not af-

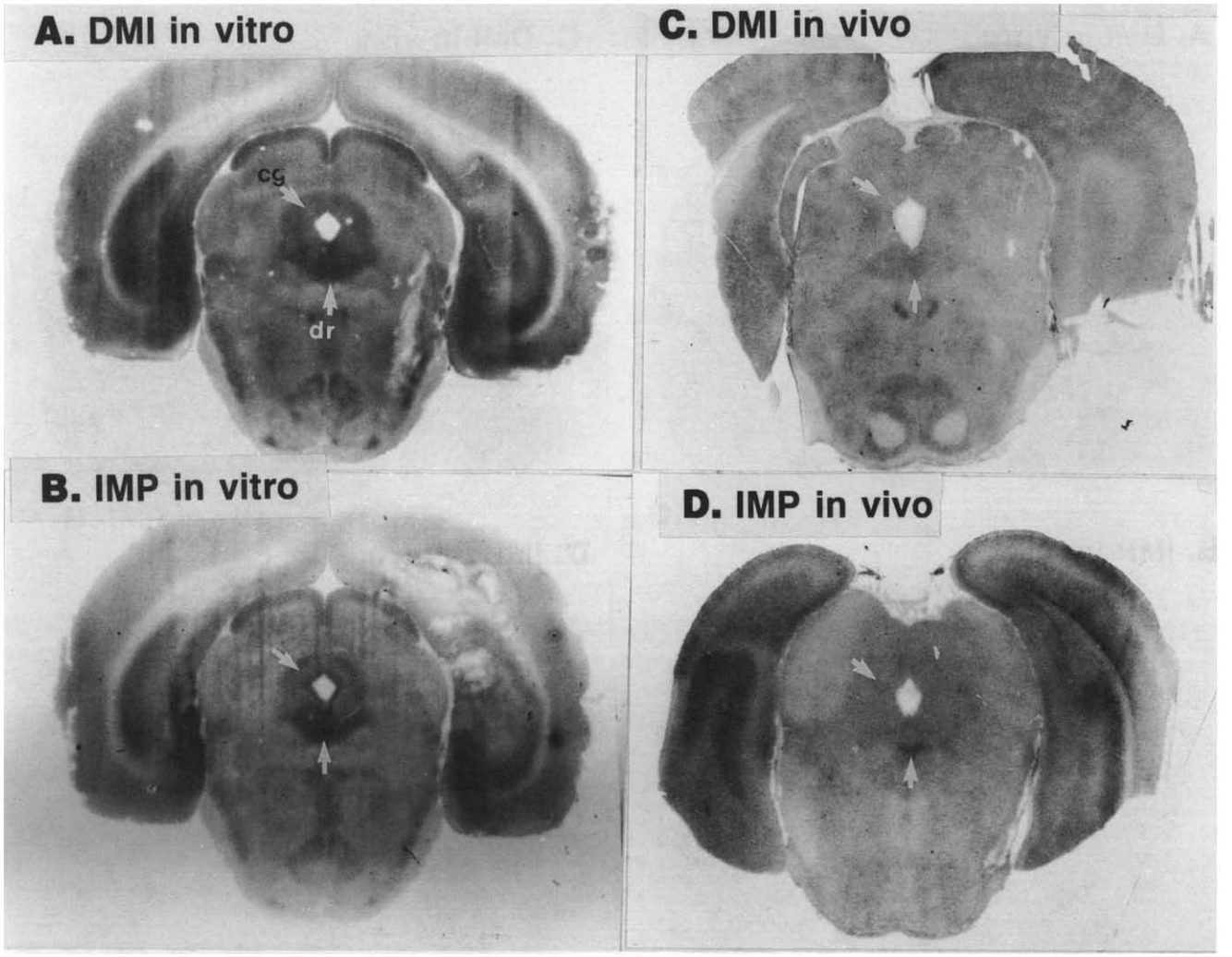

FIG. 6. In vitro binding and in vivo distribution of ^{3}H-desipramine and ^{3}H-imipramine at the level of the midbrain. Abbreviations: dr, dorsal raphe; and cg, central gray.

ter ^{3}H-desipramine. Both the parvocellular and magnocellular divisions of the paraventricular nucleus contained high amounts of radioactivity after injection of ^{3}H-desipramine, whereas only the magnocellular division was labeled for imipramine. The anterior thalamic nuclei were labeled after in vivo treatment with ^{3}H-desipramine but not after ^{3}H-imipramine. The paraventricular nucleus and anterior thalamic nuclei contain very high concentrations of noradenergic terminals (25) and may account for the association of ^{3}H-desipramine with these regions in vivo. However, other regions of dense noradrenergic innervation (25), such as the zona incerta and central nucleus of the amygdala, did not exhibit preferential localization of ^{3}H-desipramine in vivo.

The results of the present in vivo autoradiographic investigation with ^{3}H-desipramine yielded different distribution patterns compared to previous in vivo studies with this compound. In a study where radioactivity was measured in dissected brain regions 30 min after IP injection of ^{3}H-desipramine, there was no apparent regionally distinct distribution pattern (3). In an in vivo autoradiographic study, Yavin et al. (27) found that the caudate nucleus contained much higher radioactivity than other regions 30 min after IP injection of ^{3}H-desipramine. The differences between the distribution patterns described by Yavin et al. (27) and those observed in the present study are likely due to the different routes of injection and survival times employed. In the present study, rats were injected with ^{3}H-desipramine IV as a pulse, via a chronic jugular catheter, and killed 60 min after injection. At that time after IV injection, only tightly bound or sequestered compound will remain in brain. In contrast, 30 min after IP injection, more loosely bound compound may be present.

The existence of high affinity, limited capacity binding sites for ^{3}H-imipramine in many brain regions is well documented from in vitro biochemical (16,20) and autoradiographic binding studies (4,13). Based on such in vitro investigations, it could be expected that ^{3}H-imipramine would label these sites after systemic administration. However, pretreatment with unlabeled imipramine (20 mg/kg) did not reduce the amount of radioactivity in brain regions after IV injection of the radiolabeled drug. Based on these results, it was hypothesized that the dose employed (20 mg/kg) was not sufficient to saturate binding sites for the drug; therefore, a 2 dose schedule of unlabeled drug treatment was tested. Paradoxically,

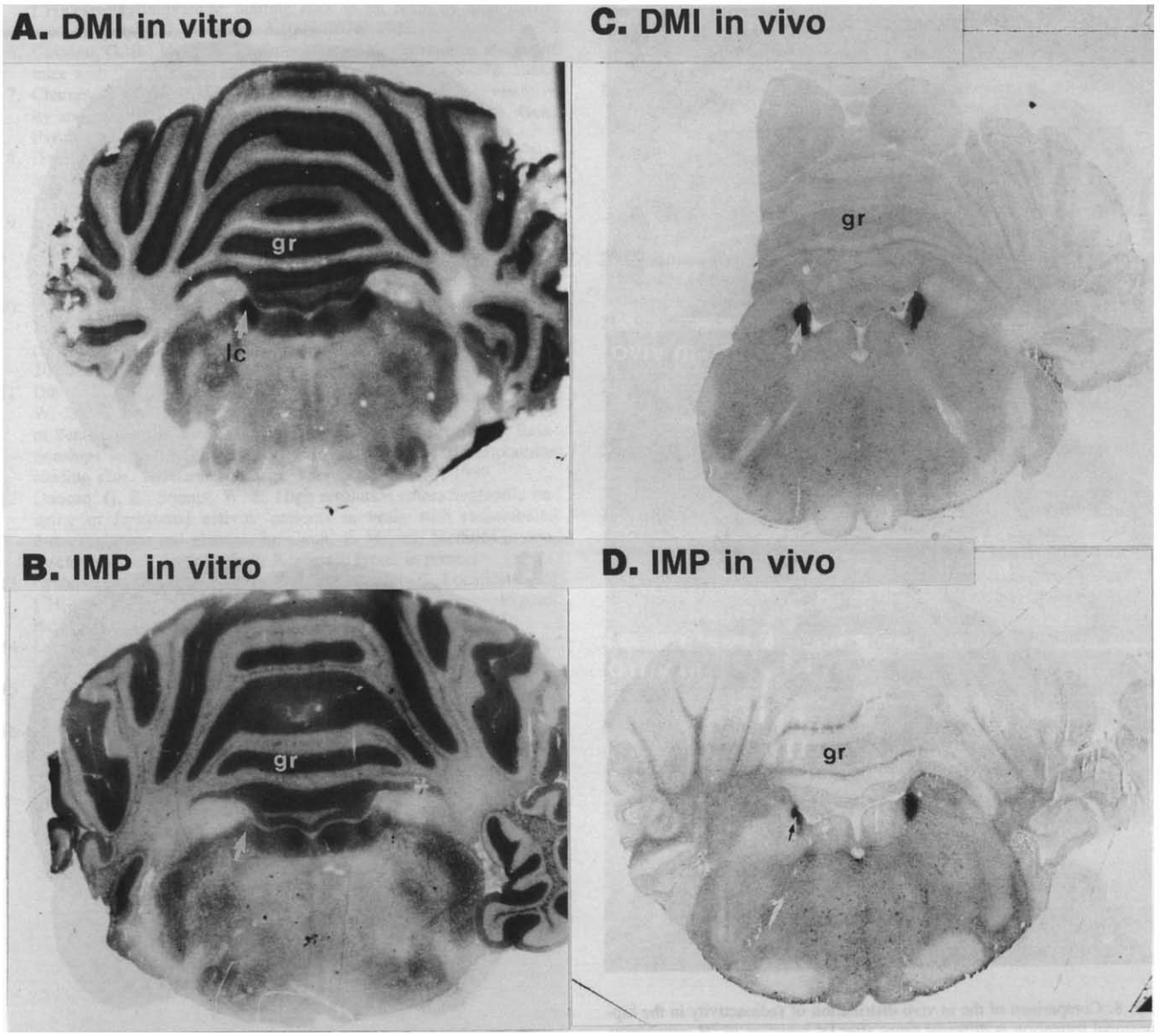

FIG. 7. In vitro binding and in vivo distribution of ^{3}H-desipramine and ^{3}H-imipramine at the level of the pons. Abbreviations: lc, locus coeruleus; gr, cerebellar granule cell layer.

after pretreatment with 2 doses of unlabeled imipramine (20 mg/kg each), a greater amount of radioactivity was observed in brain regions after IV injection ^{3}H-imipramine. These findings probably result from pharmacokinetic interactions between the labeled and unlabeled drug. For example, the high dose of the unlabeled drug may saturate plasma protein binding sites, creating a higher concentration of free ^{3}H-imipramine in plasma after IV administration. Regardless of the mechanism that accounts for increased ^{3}H-imipramine in brain after pretreatment with unlabeled imipramine, the results indicate that straight-forward interpretation of "displacement studies" with unlabeled imipramine in vivo is problematic.

Although it was not possible to displace ^{3}H-imipramine with cold imipramine in vivo, the highly selective association of the drug with discrete brain regions 60 min after IV injection argues for some specific mechanism to account for the distribution of the antidepressant drug in vivo. That imipramine influences neural mechanisms in vivo is supported by an extensive literature documenting behavioral and neurochemical effects of the drug after systemic administration (7). The high accumulation of ^{3}H-imipramine in limbic forebrain structures, including the hippocampus and amygdala, is consistent with specific pharmacological actions of the drug at these sites (1, 9, 11, 17, 18). The association of imipramine with the locus coeruleus and dorsal raphe in vivo is supportive of actions on noradrenergic and serotonergic systems, respectively. The possible relationships between the in vivo distribution of imipramine and pharmacological and therapeutic actions of the drug deserve further investigation.

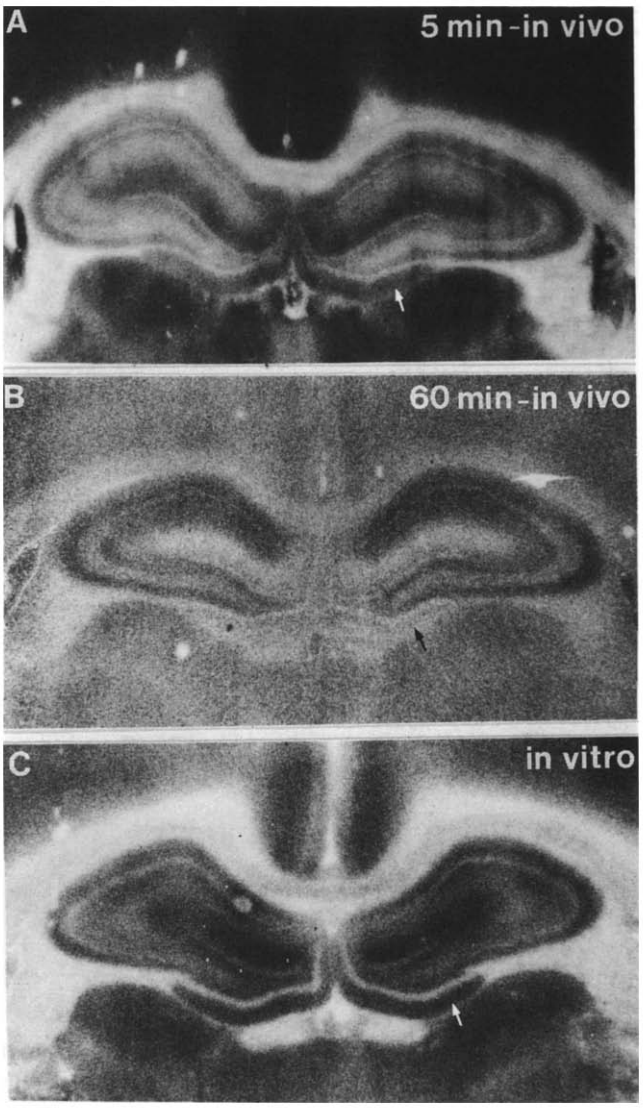

FIG. 8. Comparison of the in vivo distribution of radioactivity in the hippocampal formation at different times after IV injection of ^{3}H-imipramine (A) 5-min survival after injection (1.6 μCi/g body weight), exposure time 4 months; (B) 60-min survival after injection (1.6 μCi/g body weight), exposure time 8 months; (C) in vitro binding (5 nM, ^{3}H-imipramine) exposure time 5 months.

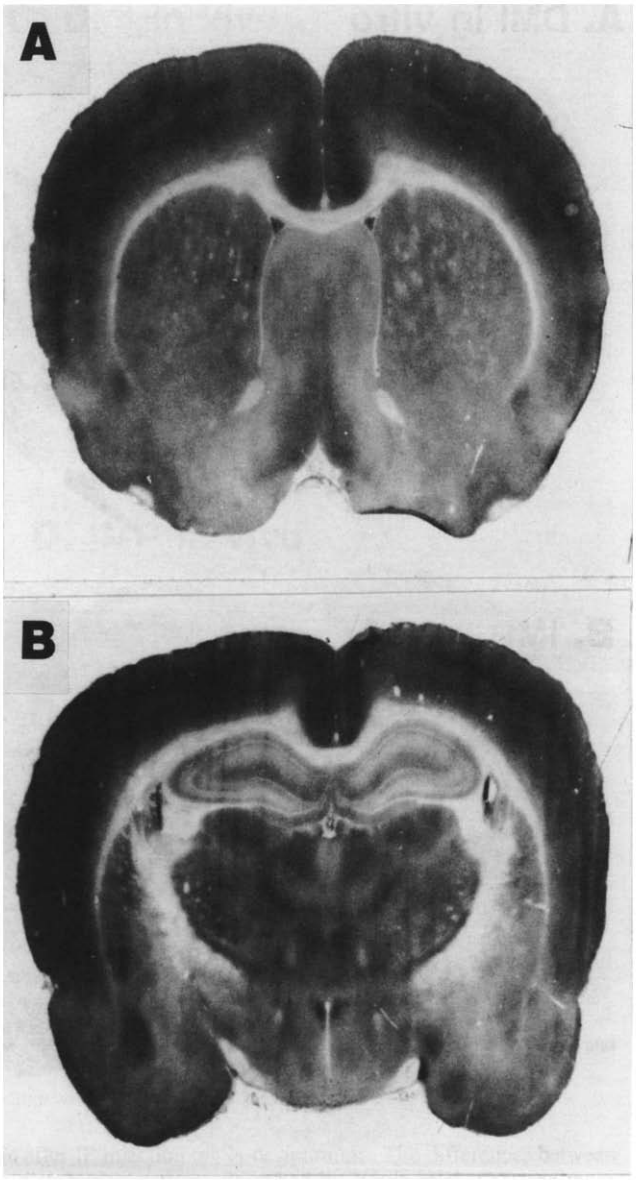

FIG. 9. Distribution of radioactivity 5 min after injection of ^{3}H-imipramine. Exposure time 4 months. The micrographs in (A) and (B) correspond to the brain levels shown in Figs. 3 and 5, respectively.

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