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Redistribution of Lidocaine and Bupivacaine after Intrathecal Injection in Mice

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Redistribution of lidocaine and bupivacaine was studied after intrathecal injection in mice qualitatively by whole-body autoradiography or quantitatively by determining spinal cord concentration of the drugs. The composition of the drug solutions then were changed to see if drug distribution could be altered. Changing the *p*H of the lidocaine solution from 7.5 to 5.0 by adding hydrochloric acid had no effect on spinal cord distribution. Simultaneous injection of bupivacaine and epinephrine increased the bupivacaine concentration in the spinal cord. By adding sucrose to a lidocaine solution, rostral spread of lidocaine was considerably less when the animals were restrained in a vertical position as compared with those injected with a plain solution. When lidocaine and bupivacaine were injected simultaneously, the spinal cord concentration was only changed to a small extent as compared with when the drugs were given separately. Whole body autoradiography revealed that both lidocaine and bupivacaine were rapidly redistributed rostrally from the lumbar area, but quantitatively the rostral redistribution is small. Whole body autoradiography also illustrated the elimination pathways from the spinal subarachnoid space. The authors conclude that alterations in the composition of the injected drug solution can affect rostral redistribution in the spinal cord tissue. (Key words: Anesthetics, local: bupivacaine; lidocaine. Anesthetic techniques: spinal. Spinal cord: subarachnoid space.)

INTRATHECAL ADMINISTRATION of drugs has common clinical applications, such as injection of local anesthetics for spinal anesthesia and of opiates for postoperative and chronic pain relief. Despite this there are very few studies on the rate and degree of redistribution of drugs after intrathecal administration or on their elimination from the spinal subarachnoid space. An indirect method that has been used is to measure plasma or blood concentration of the drug with respect to time.[1–4] This, however, does not give direct information on the behavior of the drug within the central nervous system (CNS) compartment but instead estimates the rate of elimination. Another commonly used method is to clinically map the segmental spread of spinal anesthesia and assume that this correlates with redistribution of the drug within the neuroaxis. In a study of Kitahara *et al.*,[5] ^{131}I was added to the solution and the rate of radioactive spread within the cerebrospinal fluid (CSF) was studied, but the relatively loose association of ^{131}I to the molecule and the possibility that the isotope could have altered the physical–chemical properties of the drug were confounding factors in the study. Analysis of the concentration of local anesthetics at the site of injection also has been performed,[6] but that procedure could not reveal information on the actual spread within the CNS. In a recent report we demonstrated in mice that intrathecal injection of radioactively labeled drugs could be used for studying spinal distribution and pharmacologic effects of local anesthetic agents.[7]

In clinical practice, several procedures are used to influence the spread of anesthesia. Such techniques include changing the density of the solution, adding vasoconstrictors to the solution, or choosing a drug with specifically desired physicochemical properties.[8] The true influence of these factors on the actual spread of drugs within the CNS is not known, however. The aim of the present investigation, therefore, was to measure the spinal cord concentration of drugs at different distances from the injection site, following intrathecal administration of local anesthetic solutions with varied composition. We also wanted to compare whole body autoradiographic appearance of lidocaine or bupivacaine after intrathecal injection.

Materials and Methods

ANIMALS AND DRUG ADMINISTRATION

All experiments were approved by the local ethical committee for animal experiments. Male albino rats of the NMRI strain (Anticimex, Stockholm, Sweden) weighing 20–25 g were used. The technique for intrathecal injection was essentially the same as that described by Hylden and Wilcox.[9] In brief, a small incision was made through the skin over the lumbar spinal column with the animals passively restrained on a wire mesh. A volume of 5 μl local anesthetic solution was injected into the subarachnoid space in the L5–6 vertebral interspace by lumbar puncture with a 30 G needle. The success of the in-

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jection was verified by an immediate bilateral motor blockade of the hind legs. Animals with delayed onset of block, as well as those showing unilateral blocks, were not included in the study.

Drug Solutions

For the experiments where spinal cord concentration of the drug was determined, solutions were prepared with radioactivity added as a tracer dose. Lidocaine · HCl (Astra Läkemedel AB, Sweden) was prepared at a concentration of 50 mg/ml in physiologic saline, and ^{14}C-lidocaine (NEN, Dreieich, FRG) was added to a specific activity of 50 μCi/mmol. The bupivacaine solution was prepared at a concentration of 7.5 mg bupivacaine · HCl/ml (Astra Läkemedel AB, Sweden) in physiologic saline, with ^{3}H-bupivacaine (Astra Läkemedel AB) added to a specific activity of 0.72 mCi/mmol. For whole-body autoradiography, ^{14}C-labeled lidocaine (2.2 μCi per animal, 25 μg per animal) or ^{3}H-labeled bupivacaine (75 μCi per animal, 38 μg per animal) was dissolved in 5 μl physiologic saline. When studying the effect of *p*H on distribution, the *p*H of the lidocaine solution was changed from 7.5 to 5.0 by adding HCl. The effect of a vasoconstrictor was studied by adding epinephrine bitartrate (Sigman, St. Louis, Missouri) at a concentration of 0.2 or 0.8 μg/μl to the bupivacaine solution. The baricity of the lidocaine solution was changed by adding sucrose at a concentration of 80 mg/ml. In this latter experiment, the animals were restrained in a vertical position until killed. To examine the possibility of uptake competition for local anesthetic agents in spinal cord tissue, lidocaine and bupivacaine were mixed together at a concentration of 50 mg/ml and 7.5 mg/ml, respectively.

Tissue Preparation

Following injection, animals were killed at selected times (see below) by inhalation of diethyl ether. The spinal column was dissected free and cut into 5-mm segments using a scalpel, and the segments were taken out of the spinal canal. The total length of the spinal cord was 36 ± 5 mm ($\bar{X}$ ± SEM; n = 5) from cisterna magna to the tip of the cord. Radioactive contamination between the segments was avoided by randomizing the order in which the spinal column was sectioned, and separate scalpels were used on each dissection. The exact time from drug injection to complete separation of the segments was carefully noted. The spinal cord tissues were immediately transferred to preweighed liquid scintillation vials. The weight of the tissue samples varied from 5 to 20 mg. A volume of 1.0 ml Soluene 350® (Packard Instruments) was added, and the tissue was digested at 50° C, whereafter 10 ml liquid scintillation solution (5.5 g Permablend® [Packard Instruments] per liter toluene) was added. Each sample was measured for radioactivity in a Packard 2430® liquid scintillation counter. External standard ratio was used for quench correction. The results were expressed as nanomole of local anesthetic agent per milligram wet weight of spinal cord tissue.

Whole-body Autoradiography

At 5 and 10 min, and 1 h after lidocaine injection, and at 15 min and 1 h after bupivacaine injection, the animals were anesthetized with diethyl ether and killed by complete immersion in hexane cooled to −70° C with solid CO_2. The animals subsequently were embedded in a 2.5% aqueous solution of carboxy methyl cellulose and frozen for 10 min at −70° C. Sagittal or transverse sections (20 μm) were taken at different levels of the whole body at −20° C by means of a cryomicrotome (LKB 2258 PWV, LKB-Produkter, Sweden). Each section was attached to tape (No. 810, 3 M) and dried at the same temperature for 2–3 days. The tape-mounted sections were pressed against x-ray films (Structurix® D7, Agfa-Gevaert, ^{14}C or LKB-Ultrafilm® ^{3}H (LKB 2208-191, LKB-Produkter, Sweden) and stored at −20° C for exposure (18 and 6 days, respectively). The exposed plates were developed in Agfa-Gevaert G 230 x-ray developer and fixed in Agfa-Gevaert G 305.

Statistical Calculations

For statistical analysis, the nonparametric Mann-Whitney U-test was used.[10] Statistically significant differences were defined as $P < 0.05$.

Results

Both lidocaine and bupivacaine caused very rapid flaccidity of the hindlegs after intrathecal injection. Some animals injected with bupivacaine showed a partial motor blockade of the hind legs, whereas all injected with lidocaine had a full motor blockade. The duration of the motor blockade after bupivacaine was only slightly longer than that after lidocaine, even though the concentrations of drug solutions used were chosen to resemble those used clinically for spinal anesthesia.

When the *p*H of the lidocaine solution was changed from 7.5 to 5.0 by addition of nonbuffered hydrochloric acid, it had no effect on spinal cord distribution of the drug (fig. 1). Animals were killed 5 min after injection in this experiment.

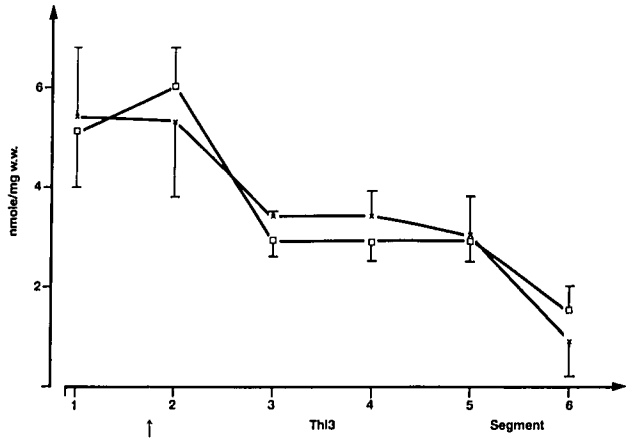

FIG. 1. Concentration (median ± quartiles; n = 5) of lidocaine in spinal cord tissue after injection of 5 μl solutions with the *p*H adjusted to 7.5 □——□ or 5.0 ×——×. The arrow indicates the site of injection (L4/L5). For further details see the text.

Epinephrine, added at a dose of both 1 or 4 μg/5 μl to the bupivacaine solution, affected the distribution of bupivacaine (fig. 2). The spinal cord concentration was thus higher at 5 and 60 min after injection of the bupivacaine solutions containing epinephrine at a dose of 1 or 4 μg/5 μl. Suprisingly, the effect was less from 4 μg than 1 μg epinephrine. The effect of epinephrine was different at 10 min, however, with a tendency towards lower tissue concentration after injection of the bupivacaine solution containing 4 μg epinephrine. Animals injected with the epinephrine-containing solution still had detectable concentrations of bupivacaine in the spinal cord at 60 min, whereas it was below the detection limit in those given the plain solution.

The redistribution of lidocaine was influenced by the addition of sucrose at a concentration of 80 mg/ml. In animals restrained vertically and administered the sucrose-containing solution, it was found that lidocaine was taken up to a lesser extent in the spinal cord as compared with those injected with the plain solution (fig. 3).

When lidocaine and bupivacaine were injected separately or in the same solution at the concentrations of 50 and 7.5 mg/ml, respectively, the only consistent tendency was a statistically significant higher concentration of bupivacaine in the spinal cord when administered together with lidocaine (fig. 4*B*). Lidocaine was present in statistically significant higher concentration in the most cephalad segment when injected separately than in combination (fig. 4*A*).

The whole-body autoradiography showed that bupivacaine persisted in comparatively low concentrations in the brain and brain stem 15 min. after injection, compared with the intense labeling of the lumbar spinal cord close to the site of injection (fig. 5*A*). The cord was labeled more intensely in the dorsal than ventral parts in the mid-

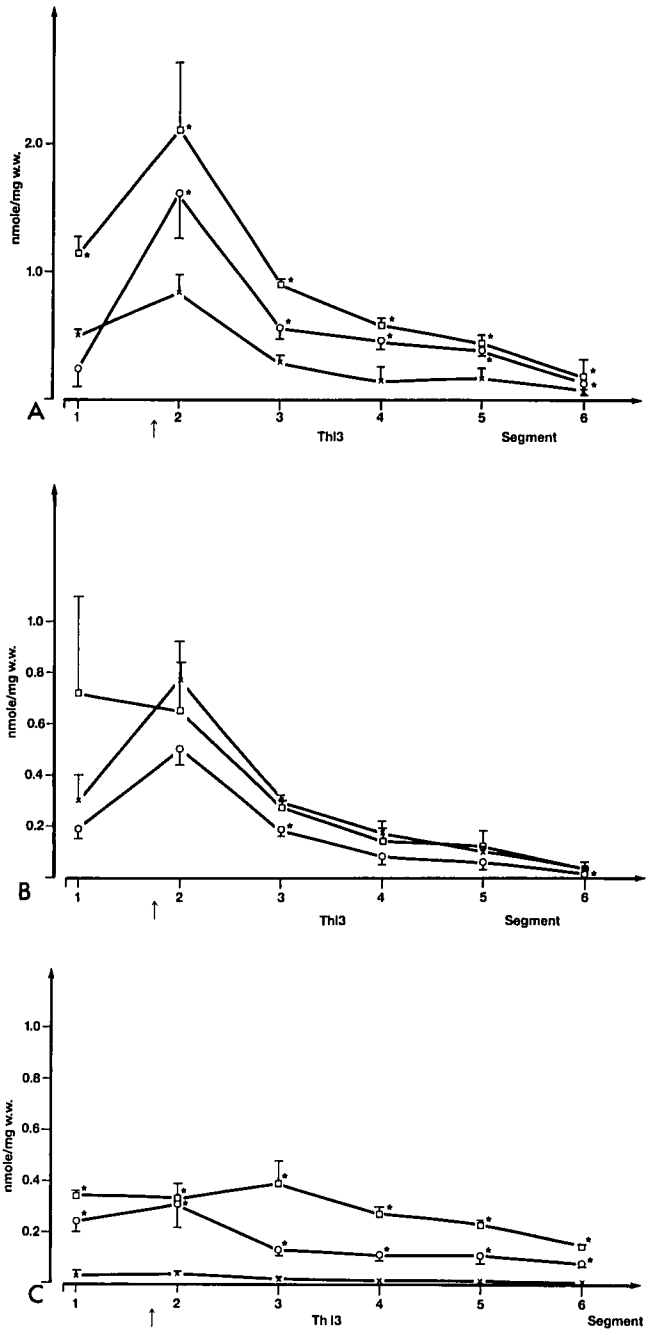

FIG. 2. Concentration of bupivacaine (median ± quartiles; n = 5) in spinal cord tissue after injection of solutions with 7.5 mg/kg bupivacaine alone ×——×; with epinephrine added at a dose of 1 μg □——□ or 4 μg ○——○. *A*. Five minutes after injection. *B*. Ten minutes. *C*. Sixty minutes. **P* < 0.05 (Mann-Whitney U-test) when comparing solutions with vasoconstrictor to the plain solution.

thoracic area. The liver and gastrointestinal tract were visible already at 15 min after injection, and radioactivity also was seen in the urinary bladder. At 1 h after injection, no further rostral transport seems to have occurred (fig. 5*B*). By this time, the local anesthetic effect of bupivacaine had disappeared and the drug had now redistributed to a larger extent to the urine. The activity in the liver was considerably lower than that at 15 min. At 1 h, there was detectable activity in the spinal cord and the cauda equina. At 5 min after injection of lidocaine, the brain and brainstem contained very low levels of radioactivity, compared with that in the thoracic and lumbar spinal cord (fig. 6*A*). The spinal cord was densely labeled in the lumbar region, whereas in the cervical portion it had comparatively low levels of radioactivity. The elimination via the venous return from the spinal column readily could be seen. Already by this time, the urinary bladder was visible. The outer part of the lumbar cord was still more labeled than the inner at 10 min after injection (fig. 6*B*). In the thoracic cord, the radioactivity was less dense than at 15 min after the injection. The liver and urinary bladder had higher levels of radioactivity than that at 5 min. No detectable activity appeared in the spinal cord at 1 h (fig. 6*C*), but the gall bladder, gastrointestinal tracts, and urinary bladder were still readily observed. When the animals were sectioned in a transverse direction 5 min after injection, there was a core without activity at the site of injection (fig. 7*B*). The radioactive trace observed ventrally of the spinal column fits with a blood vessel appearing on the tissue section. The activity on the dorso-lateral surface of the vertebral body was found to correspond to the external vertebral plexus serving the venous return from the spinal cord to the systemic venous system. The marginal parts, and especially the dorsal regions of the cord in the midthoracic spinal cord 21 mm rostral to the site of injection, were labeled at 5 min (fig. 7*A*), and the activity declined toward the center. The activity in CSF was lower than that of the outer parts of the spinal cord. On an autoradiograph taken from a section 10 mm caudal to the injection site (fig. 7*C*), elimination via the venous return was observed as readily as at the injection site (cf. fig. 7*B*). The vertebral canal was heavily labeled in the caudal section, and, when examining the corresponding tissue slice, it was found that it at this level was filled by the spinal nerves of the cauda equina. The individual nerves could not be detected on the autoradiograph, however.

Discussion

The present investigation demonstrates differences in redistribution of intrathecally injected local anesthetic agents by measuring spinal cord tissue concentrations along the neuroaxis. We have thus found that this can be affected by varying the composition of the injected drug

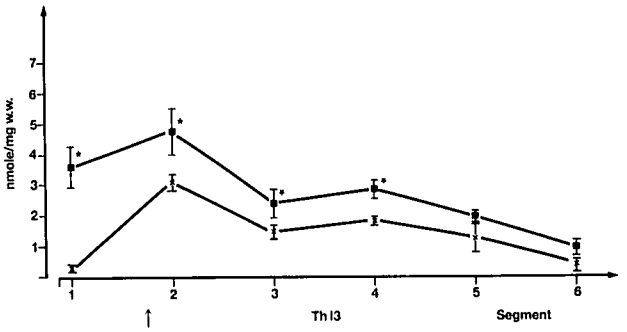

FIG. 3. Concentration of lidocaine (median ± quartiles; n = 4) in spinal cord tissue 7 min after injection of solutions with varying density. × —— × animals injected with solutions made hyperbaric by adding sucrose at a concentration of 80 mg/ml and restrained in a vertical position until killed; ■ —— ■ animals injected with a plain solution and kept vertically until killed. *$P < 0.05$ (Mann-Whitney U-test).

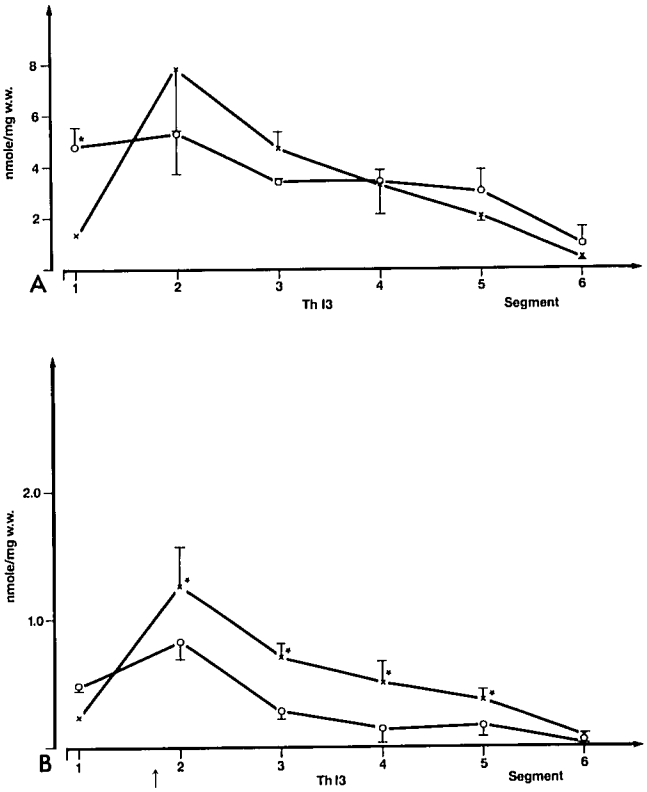

FIG. 4. Spinal cord tissue concentration of lidocaine (*A*) and bupivacaine (*B*) (median ± quartiles) after injecting a solution containing lidocaine (50 mg/ml) and bupivacaine (7.5 mg/ml) alone O —— O or as combinations × —— ×. *$P < 0.05$ (Mann-Whitney U-test).

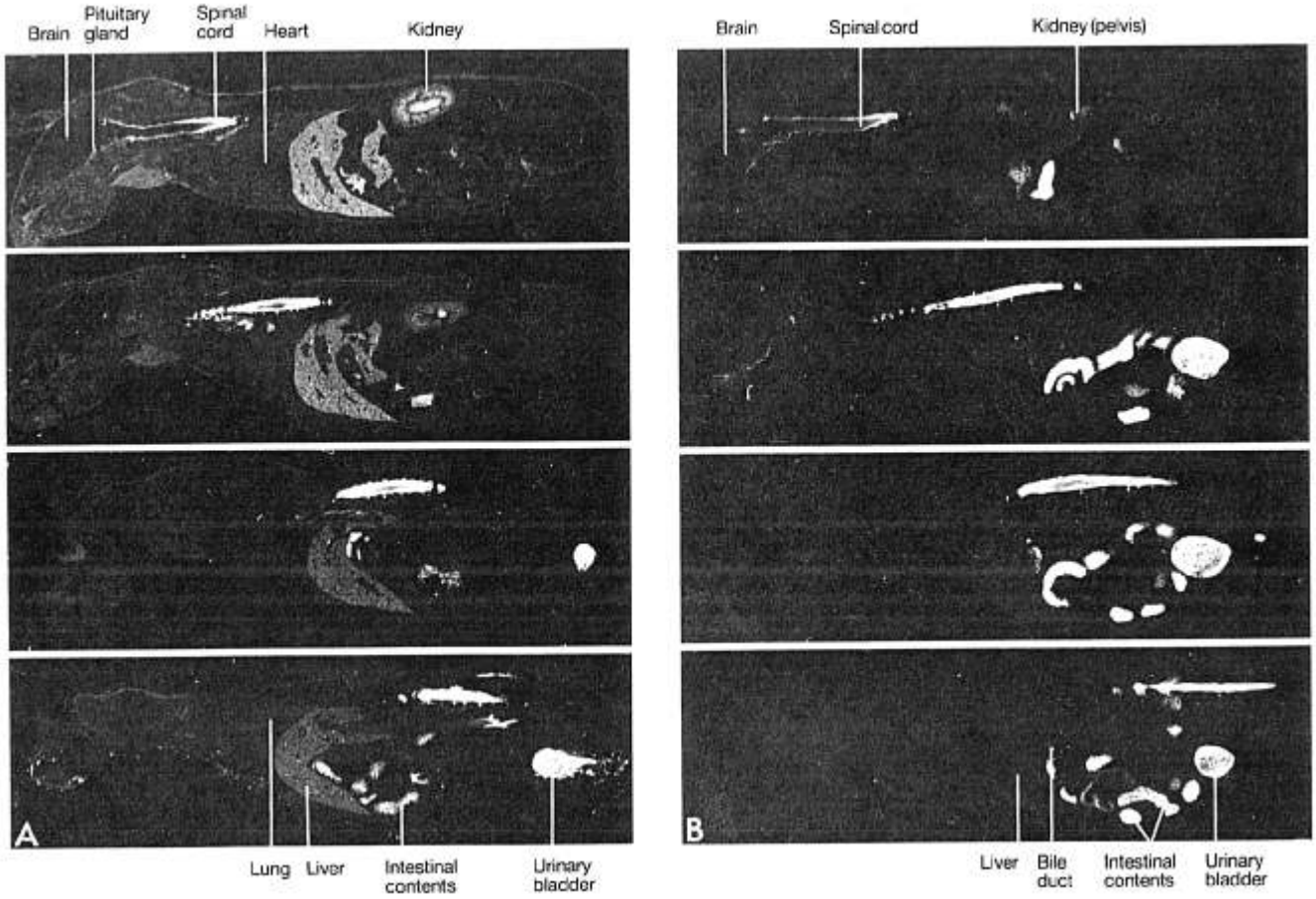

FIG. 5. Autoradiogram showing distribution of bupivacaine in a mouse after intrathecal injection of 38 μg bupivacaine (75 μCi) in a volume of 5 μl at 15 min (*A*) and 1 h (*B*). The individual sections are from the same mouse, mounted together to show the distribution of activity along the whole CNS.

solution. The whole-body autoradiographic appearance of the animals at various times after injection also has been investigated and reveals the patterns of CNS redistribution and elimination therefrom.

A critical point in these experiments is whether the doses and volumes injected are relevant to the clinical use of the drugs. If the volume is extrapolated to a person weighing 70 kg, the equivalent would be approximately 10 ml. A patient given 10 ml intrathecally would, however, develop an almost complete spinal blockade, whereas the animals in the present study all had the motor blockade restricted to the hind legs only. Furthermore, they showed no signs of supraspinal effects such as sedation or respiratory depression. These results and the unexpected low degree of rostral redistribution supports the conclusion that 5 μl in the mouse appears to be an adequate volume for studying redistribution of intrathecally administered drugs. We have previously shown, furthermore, that when the injected volume was increased from 5 to 10 μl, there was a more rostral redistribution of the local anesthetic agent.[7]

It has been proposed that the tachyphylaxis observed after several doses of local anesthetic agents given epidurally with slightly acidic solutions could be due to saturation of the buffering capacity of the CSF.[11] In the present study, a considerable change in *p*H from 7.5 to 5.0 did not affect the distribution of lidocaine, however. This is a surprising finding, as the degree of charge of lidocaine by this change in *p*H is shifted from 61% to less than 1%, as the pK_a is 7.7.[12] The *p*H was adjusted with hydrochloric acid, however, and not with a buffered solution. The buffering capacity of the CSF did probably therefore neutralize the acidic drug solution. It would be interesting to extend this experiment over a larger range of *p*H values and to use solutions with stronger buffering capacity than that in the present study.

The spinal cord concentration was higher at 5 min after injection when epinephrine was added to the solution. At 10 min, however, there were very small differences between the groups. The animals injected with the solution containing 4 μg epinephrine even showed a statistically significant lower spinal cord concentration of bupivacaine than those injected with the plain solution. The animals injected with the epinephrine-containing solutions had higher concentrations of bupivacaine in the spinal cord at 60 min than those given the plain solution (fig. 2*C*). In clinical practice it has recently been reported that local anesthetic solutions containing vasoconstrictors only give rise to small differences in the time of onset or the duration of both motor and sensory block.[13] In a study by Axelsson and Widman,[3] it was demonstrated that patients given lidocaine with epinephrine had a slight prolongation of the sensory block and that the blood concentrations were lower up to 120 min after injection. Denson *et al.*,[14] on the other hand, reported that in monkeys there were no differences in duration of complete motor or sensory blockade produced by lidocaine when epinephrine was added to the solution. The time to complete recovery was longer with epinephrine, however. It was also demonstrated that there were no differences in rate of absorption or other pharmacokinetic parameters between the two groups. The finding in the present investigation that the animals injected with the solution containing epinephrine still at 60 min had higher tissue concentrations of bupivacaine is interesting. It is not in conflict with the study by Denson *et al.*,[14] as less than 10% of the drug retained in the spinal cord probably would not have been detected by the pharmacokinetic model. This could mean that the longer time to full recovery observed after administering local anesthetic solutions with vasoconstrictor[3,14] could be due to the local anesthetic agent still present in the neural tissue but at concentrations not giving rise to a full local anesthetic effect. The effect of such concentrations in the spinal cord could be a blockade of synaptic transmission[15] rather than the axonal impulse propagation.

The addition of sucrose to the local anesthetic solution for restricting the rostral spread of the drug also was assessed in the present investigation. It was found that when lidocaine was injected as a hyperbaric solution, there was less rostral redistribution of the drug when the animals were restrained in a vertical position, as opposed to those administered the plain solution (fig. 3). These results thus support the notion that hyperbaric solutions produce less rostral spread when the patient is kept supine immediately after injection and that this clinical observation[16] corresponds to effects on spinal cord concentrations. Figure 3 indicates that injection of the hyperbaric lidocaine solution restricts the total amount of local anesthetic agent present in the tissue. The most probable reason for this effect is that the solution is deposited in the dural sac below the spinal cord and therefore is not available for tissue uptake.

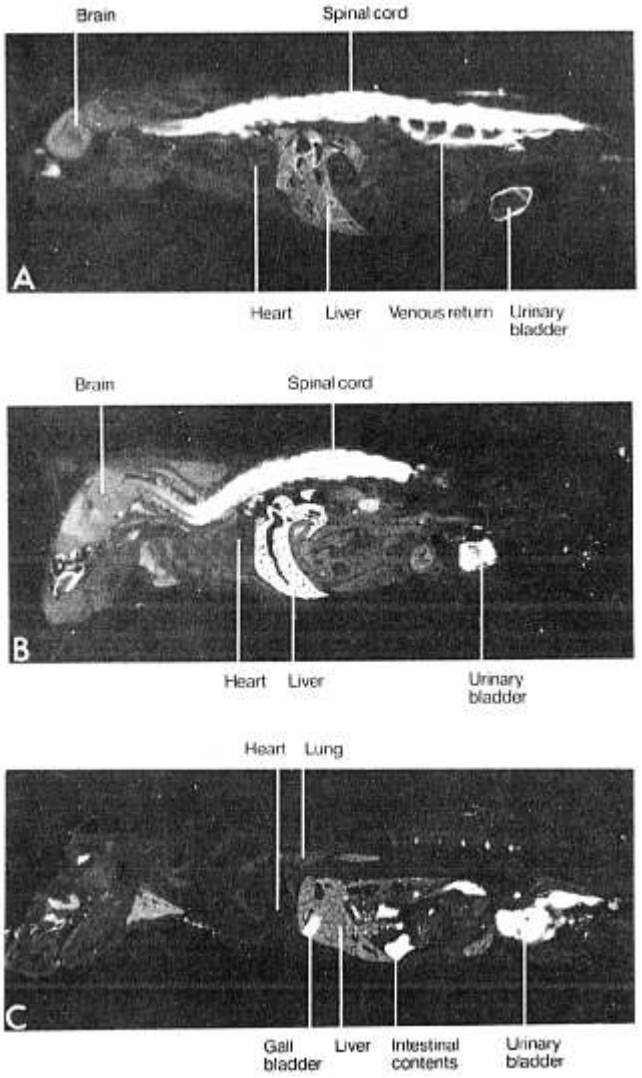

FIG. 6. Autoradiogram showing distribution of lidocaine in mice after intrathecal injection of 25 μg lidocaine (2.2 μCi) in a volume of 5 μl. The animals were killed at 5 min (*A*), 10 min (*B*), and at 1 h (*C*).

Competition for binding has been shown to occur in other than nervous tissue, such as lung parenchyma,[17] where basic amine drugs share common binding sites of low capacity. The only statistically significant difference in tissue concentration in the present study was an increased level of bupivacaine when injected together with lidocaine (fig. 4). No obvious mechanism can be proposed for this effect, however. Further studies should be carried out to investigate these effects, as simultaneously administered drugs into the intrathecal space probably will become increasingly common in clinical practice.

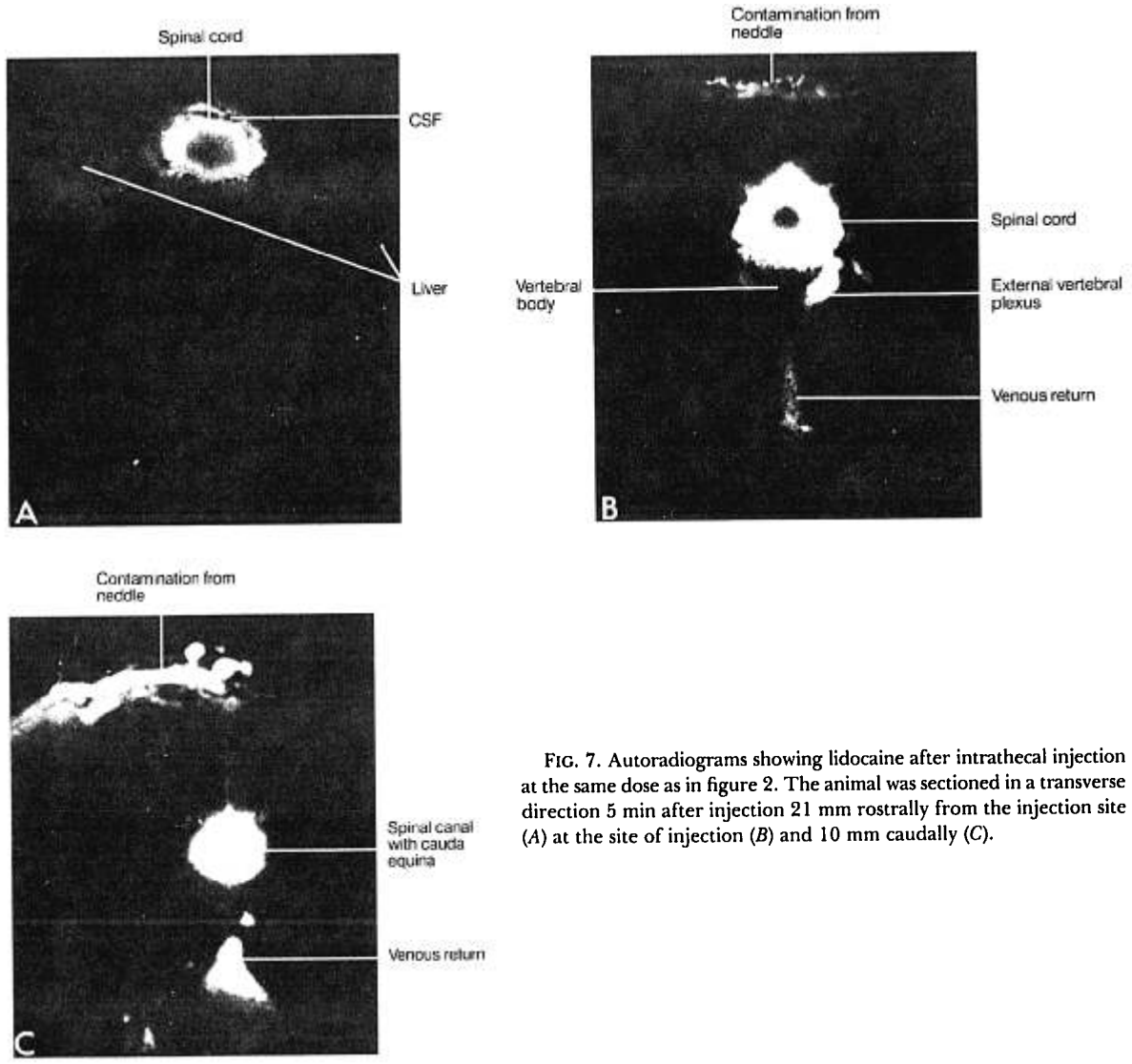

FIG. 7. Autoradiograms showing lidocaine after intrathecal injection at the same dose as in figure 2. The animal was sectioned in a transverse direction 5 min after injection 21 mm rostrally from the injection site (*A*) at the site of injection (*B*) and 10 mm caudally (*C*).

The results from the whole-body autoradiography confirm that there is a correlation between the pharmacologic effects of the local anesthetic agents and the CNS distribution. Thus, in the cervical portion of the cord, there was less radioactivity as compared with that close to the site of injection, and the motor function of the front legs were unaffected. Both in the longitudinal autoradiograph at 15 min after bupivacaine (fig. 5*A*), at 10 min after lidocaine (fig. 6*B*), and in the transverse autoradiographs after lidocaine (figs. 7*A–C*), it was apparent that the dorsal portions of the cord had a higher level of radioactivity. This finding is in agreement with that obtained in dogs by Cohen.[18] The elimination from the spinal subarachnoid space could be readily seen in both the longitudinal and transverse autoradiographs. In the longitudinal sections, the extravertebral plexuses appeared as regular spines of activity, one over each intervertebral space, collecting into the caval vein (*e.g.*, fig. 5*A*). This elimination appeared even more clearly in the enlarged transverse section.

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