3

Preclinical Aspects of Local Anesthesia

IN VITRO STUDIES

The concentration of drug required to inhibit nerve conduction has been used to define the intrinsic anesthetic potency of a chemical substance. In an attempt to compare the relative potency of different anesthetic agents, the concept of minimum anesthetic concentration (Cm) was introduced and defined as the minimum concentration of local anesthetic agent necessary to block impulse conduction in a nerve fiber of given diameter within a reasonable period of time.[1] Cm was intended to be comparable to the minimum alveolar concentration (MAC) of general inhalation anesthetic agents and so could serve as a means of grading relative potency. Unfortunately, differences in experimental techniques and definitions make it impossible to compare the Cm values as reported by various investigators.

Experimental Models

In vitro isolated nerve preparations have been employed by most investigators to determine the conduction blocking activity of specific chemical compounds. Although the electrode configuration used may differ from laboratory to laboratory, the type of apparatus commonly utilized to study nerve conduction consists essentially of a series of electrodes on which an isolated nerve can be placed (Fig. 3-1).[105–107] One segment of the nerve can be stimulated electrically with square wave pulses of varying intensity, duration, and frequency, and surface action potentials can be recorded from the opposite end. A

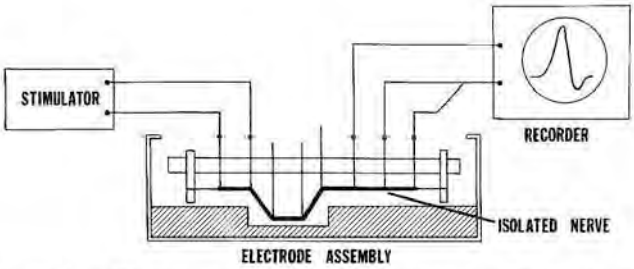

Fig. 3-1. Diagram of experimental apparatus for isolated nerve studies.

segment of the nerve between the stimulating and recording electrodes is bathed with the local anesthetic solution. The sequential electrical events which occur following exposure of that segment of the nerve to local anesthetic agents consist of (1) a prolongation of the time interval between the stimulus artifact and the action potential, which indicates delayed conduction through the bathed segment and (2) a decrease in the height of the action potential, which indicates complete block of conduction in individual nerve fibers (Fig. 3-2).[108] Most studies have utilized the local anesthetic concentration required to reduce the amplitude of the surface action potential within a specific time period as a measure of intrinsic anesthetic potency. How-

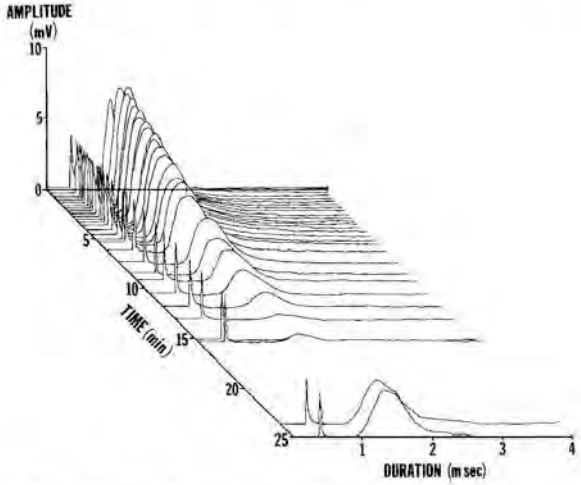

Fig. 3-2. Three dimensional action potential response of isolated frog sciatic nerve to 5 m*M* lidocaine.[108] (Courtesy of Dr. Meymaris.)

ever, markedly different potency values have been reported for the same agent depending upon the desired reduction of the amplitude of the action potential and the desired "specific time". For example, the reported concentrations of lidocaine which will produce a significant depression of the action potential amplitude in an isolated nerve preparation vary from 0.25 to 20 m*M*,[20,41,71,76,97,107,109,110] depending on the end point of amplitude depression, time of exposure to local anesthetic solution, pH of bathing solution, intensity and frequency of nerve stimulation, and type of nerve preparation.

The degree of depression of the action potential amplitude selected to evaluate anesthetic activity can markedly alter the relative Cm values. A 50% suppression in action potential height of the sheathed frog sciatic nerve can be achieved by 5 minutes exposure to 5 m*M* lidocaine. An 80% depression requires 10 m*M* and complete suppression is achieved within 5 minutes with 20 m*M* lidocaine (Fig. 3-3). If the degree of amplitude depression is maintained constant and the time required to produce the desired reduction is altered, then the

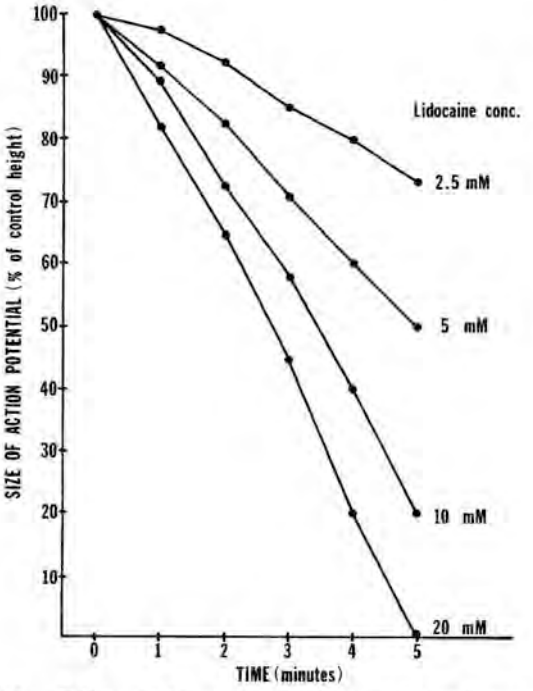

Fig. 3-3. Effect of local anesthetic concentration on rate of conduction blockade.

Cm value again will vary. A 50% suppression in amplitude can be achieved in 5 minutes by the use of a 5 m*M* concentration of lidocaine. However, only 2.5 m*M* of this agent are required to produce the same degree of depression in 10 minutes.

The pH of the bathing solution will influence the Cm value of certain local anesthetic agents.[71, 72, 76, 78, 97] A 50% reduction in amplitude of the desheathed rabbit vagus nerve can be achieved within 10 minutes with 10 m*M* of dibucaine solution at a pH of 7.2. One hundred m*M* dibucaine is required to produce the same action potential suppression when the pH of the bathing solution is 9.2.[72] This alteration of anesthetic potency by pH is related to the pK_a of the chemical compound and the proportion of drug present in the base and cationic form (Fig. 3-4). As indicated in Chapter 2, the cationic

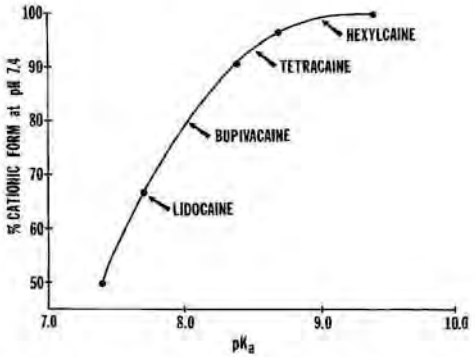

Fig. 3-4. Relationship of pK_a to percent of local anesthetic agent present in cationic form at pH 7.4.

form of the class D agents, e.g., lidocaine, mepivacaine, is mainly responsible for local anesthetic activity. Therefore, a reduction in pH will produce an increase in the proportion of available cation, which, in turn, will result in an apparent increase in anesthetic potency on desheathed nerves.

The action potential usually recorded from an isolated nerve represents mainly the A spike of the compound action potential. The height of the A spike will vary as a function of the stimulus intensity until a maximum amplitude is achieved indicating all A fibers are firing. The stimulus intensity should be adjusted initially to produce an action potential of maximum height, i.e., the stimulus intensity should be supramaximal. Changes in stimulus intensity must be avoided during the period of exposure to the local anesthetic solution

in order to ascertain accurately the conduction blocking effect of the anesthetic agent.

The rate of stimulation will also affect the apparent anesthetic potency (Fig. 3-5). When the stimulus frequency is increased from 3 to 30 pulses/sec, 5 m*M* lidocaine decreases the amplitude of the A spike to approximately 60% and 50% of its initial height respectively. At a stimulus frequency of 100 pulses/sec, 5 m*M* of lidocaine will reduce the A spike of the sheathed frog sciatic nerve to approximately 40% of its control height within 5 minutes.

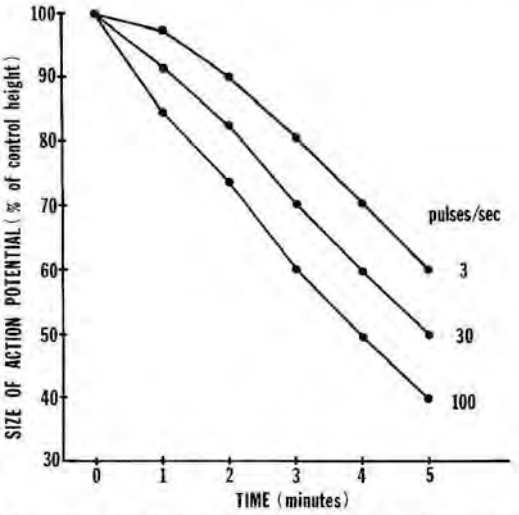

Fig. 3-5. Effect of frequency of stimulation on rate of conduction blockade.

The type of nerve preparation also will influence the observed intrinsic potency. In addition to the frog sciatic nerve, which has been employed most commonly for the in vitro study of anesthetic activity, mammalian nerves such as the rabbit vagus also have been utilized. A comparison of the desheathed frog sciatic nerve and desheathed rabbit vagus nerve reveals a more rapid decrease and a more profound depression of the frog sciatic nerve action potential than of the rabbit vagus following exposure to 300 μM of lidocaine. Moreover, this same concentration of lidocaine was found to exert a much greater depressant effect on the B fibers of the sheathed rabbit vagus nerve as compared to its A fibers.[71] Heavner and de Jong observed that preganglionic B fibers from the rabbit cervical sympathetic trunk, although myelinated, were three times more sensitive to lidocaine than

unmyelinated postganglionic C fibers.[111] These findings may have clinical relevance since sympathetic blockade (B fibers) is more extensive than sensory block (C fibers) following spinal anesthesia.

Finally, the presence or absence of the nerve sheath can alter significantly the apparent anesthetic potency of different agents. A desheathed nerve preparation is considerably more sensitive to the conduction blocking action of the commonly used clinical agents such as lidocaine and bupivacaine at a neutral pH.[71, 79] The clinical application of this phenomenon can be appreciated from a comparison of the local anesthetic dosage requirements for spinal and epidural anesthesia. Only 50–100 mg of lidocaine will produce profound anesthesia when administered into the subarachnoid space where the spinal cord is comparable to a desheathed nerve preparation. In the epidural space, where sheathed nerve fibers are present, 200–300 mg of lidocaine are required to attain sensory analgesia similar in quality to spinal anesthesia.

Intrinsic Potency

The commonly used local analgesic agents have been evaluated by determining the minimum concentration (Cm) required to produce a 50–60% reduction in A spike amplitude of the sheathed frog sciatic nerve within 5 minutes in a solution of pH 7.2–7.4 and at a stimulus frequency of 30 pulses/sec. Under these standardized conditions, procaine is the least potent of the agents currently used in clinical practice (Table 8). Chloroprocaine, lidocaine, mepivacaine, and

Table 8

RELATIVE IN VITRO CONDUCTION BLOCKING AND PHYSICAL-CHEMICAL PROPERTIES OF VARIOUS AGENTS

AGENT	RELATIVE CONDUCTION BLOCKING PROPERTIES*			PHYSICAL-CHEMICAL PROPERTIES		
	POTENCY	ONSET	DURATION	pK_a	LIPID SOLUBILITY	PROTEIN BINDING
Low Potency						
PROCAINE	1	1	1	8.9	0.6	5.8
Intermediate Potency						
MEPIVACAINE	2	1	1.5	7.6	1.0	77
PRILOCAINE	3	1	1.5	7.7	0.8	55
CHLOROPROCAINE	4	0.8	0.75	8.7	—	—
LIDOCAINE	4	0.8	1.5	7.7	2.9	64
High Potency						
TETRACAINE	16	2	8	8.5	80	76
BUPIVACAINE	16	0.6	8	8.1	28	95
ETIDOCAINE	16	0.4	8	7.7	141	94

*Data derived from isolated frog sciatic nerve

prilocaine may be classified as compounds of intermediate potency, i.e., 2–4 times as potent as procaine. Tetracaine, bupivacaine, and etidocaine represent drugs of high potency which are approximately 20 times more active than procaine. Tetrodotoxin is the most potent conduction blocking substance studied to date. Eighty nmoles of this compound will produce a 50% reduction in the amplitude of the surface action potential of the desheathed rabbit vagus nerve.[112, 113] This would indicate that tetrodotoxin has an intrinsic anesthetic potency which is approximately 250,000 times greater than that of procaine.

Onset of Conduction Blockade

One of the important clinical parameters in regional anesthesia is the rapidity with which conduction blockade occurs. This pharmacological property of local anesthetic agents can be evaluated quite accurately in an isolated nerve preparation. The relative latency of the clinically useful analgesic agents has been determined in a standardized manner, i.e., the concentration required to produce a 50% reduction in the height of the sheathed frog sciatic nerve action potential within 10 minutes. A correlation was believed to exist between the potency, duration, and onset time of local anesthetic agents. Agents of high potency and long duration of action such as tetracaine and dibucaine have historically shown the slowest onset of anesthesia.[20] However, the introduction of newer agents of high potency and long duration of action such as bupivacaine and etidocaine indicates that no correlation exists between the onset of action of various chemical compounds and their intrinsic anesthetic potency or duration of activity.[43] In fact, etidocaine possesses the most rapid onset time and tetracaine shows the longest latency (Table 8). Onset time is probably related to the physicochemical properties of these various agents, e.g., pK_a and lipid solubility. For example, a comparison of agents with similar pK_a values such as lidocaine, prilocaine, and etidocaine reveals that the most lipid-soluble drug, etidocaine, demonstrates the most rapid onset of action whereas the least lipid-soluble agent, prilocaine, has the longest latency. Lidocaine occupies an intermediate position both in terms of lipid solubility and onset time. A comparison of highly lipid-soluble compounds with varying pK_a values, e.g., etidocaine, bupivacaine, and tetracaine, indicates that etidocaine, which possesses the lowest pK_a, has the most rapid onset of action, whereas tetracaine possesses the highest pK_a value and the slowest onset time. The pK_a and latency values for

bupivacaine lie between the two extremes.[20, 114, 115] These observations concerning onset time are consistent with the known relationships between pH, pK_a, relative proportion of analgesic agents in the base and cationic form, lipid solubility, and lipid composition of the cell membrane.

Duration of Action

In an isolated nerve preparation, conduction blockade will persist as long as the nerve remains exposed to the local anesthetic containing solution. Duration of action in vitro usually is determined in the following manner: following exposure of an isolated nerve for a period sufficient to reduce the surface action potential by 50%, the local anesthetic solution is removed. The nerve then is bathed with a normal Ringer's solution. The time required for the action potential to return to its control amplitude is a measure of the duration of action of the local anesthetic agent. It is necessary to use equipotent concentrations of local anesthetic drugs when comparing their relative durations of action, since the duration of conduction blockade with any single agent varies as a function of the anesthetic concentration of that agent.[116] The clinically useful analgesic drugs can be classified essentially into two categories based on their in vitro durations of action: agents of short duration, i.e., 15 to 30 minutes are required for complete recovery, and agents of long duration, i.e., 100 to 150 minutes are required for complete recovery. Procaine, chloroprocaine, lidocaine, mepivacaine, and prilocaine fall into the first category whereas tetracaine, bupivacaine, and etidocaine are agents possessing a long duration of action. In general, those compounds belonging to the short-duration class possess relatively low lipid solubility and low protein-binding characteristics. Agents of long duration are characterized by high lipid solubility and high protein-binding properties (Table 8).

IN VIVO ANIMAL STUDIES

Models

A number of animal models have been utilized to evaluate the pharmacological profile of local anesthetic agents in vivo.[107] Intradermal wheals have been performed mainly in guinea pigs as an example of infiltration anesthesia.[12, 88, 116-119] Sciatic nerve blocks in

rats and brachial plexus blocks in guinea pigs have been employed for peripheral nerve blockade.[12, 88, 90, 106, 110, 119] In recent years, techniques for producing epidural anesthesia in guinea pigs, cats, dogs, and sheep have been developed.[90, 120, 121] In addition, spinal anesthesia in rabbits, dogs, and sheep has been utilized as a model for central neural blockade.[110, 122–124] Local anesthetic solutions have been applied to the rabbit cornea and instilled intratracheally in rabbits to evaluate the topical anesthetic activity of various compounds.[106, 110, 119] With these various techniques, it is possible to determine frequency of anesthesia, onset time, and duration of action.

Anesthetic Potency

The in vivo anesthetic potency of local anesthetic agents may differ from their intrinsic potency as determined by in vitro techniques. This may be due to other factors such as vasodilation and/or physiological disposition. For example, the intrinsic potency of chloroprocaine is approximately 4 times greater than that of procaine in an in vitro system (Table 9). However, in vivo studies

Table 9

COMPARATIVE INTRINSIC ANESTHETIC POTENCY AND IN VIVO Cm OF VARIOUS LOCAL ANESTHETIC AGENTS

AGENT	RELATIVE INTRINSIC POTENCY	Cm* RAT SCIATIC NERVE	Cm** CAT EPIDURAL ANESTHESIA
PROCAINE	1	1.0	4.0
MEPIVACAINE	2	0.5	2.0
PRILOCAINE	3	0.5	2.0
CHLOROPROCAINE	4	1.0	2.0
LIDOCAINE	4	0.5	2.0
BUPIVACAINE	16	0.125	0.5
ETIDOCAINE	16	0.125	0.5
TETRACAINE	16	0.125	0.5

*Cm = minimum concentration (0.2ml) required to produce 50% anesthetic frequency.
**Cm = minimum concentration (1.5ml) required to produce 50% frequency of flexor reflex blockade.

suggest that the equieffective concentration of these two agents is the same. This finding may be related to the extremely rapid hydrolysis of chloroprocaine as compared to procaine. Similarly, the intrinsic potency of lidocaine has been shown to be approximately 1.5–2 times greater than that of mepivacaine and prilocaine (Table 9), whereas animal data indicate that the concentrations of these three agents required to produce equivalent anesthetic activity are similar when

used for sciatic nerve blocks in rats or spinal anesthesia in rabbits.[88, 110] This apparent discrepancy between in vivo and in vitro anesthetic potency may be attributable to a greater vasodilator action of lidocaine, which results in a more rapid vascular absorption and decreased availability of this agent for neural uptake. With regard to compounds in the high potency group, bupivacaine and etidocaine possess the same Cm in terms of conduction blockade in the isolated frog sciatic nerve preparation.[114, 115] However, epidural studies in sheep indicate that 1% etidocaine is required to produce anesthetic results comparable to those achieved with 0.75% bupivacaine.[121] This difference may be due to the greater lipid solubility of etidocaine, which causes this agent to be sequestered in peridural fat with fewer molecules available for neural uptake. A comparison of the relative in vitro and in vivo anesthetic potency of the various clinically useful agents is presented in Table 9.

Topical anesthetic potency may differ from the in vivo anesthetic potency which is obtained following injection of agents in the region of nerve endings or fibers. Block of the corneal reflex in rabbits and guinea pigs has been used most frequently to evaluate the topical anesthetic properties of different agents.[107] Other techniques such as blockade of the sneeze reflex in rabbits have also been employed.[110] A comparison of the topical anesthetic potency of various local anesthetic compounds as evaluated in animal preparations indicates that tetracaine and cocaine demonstrate the most potent topical anesthetic activity, followed in order of decreasing potency by etidocaine, bupivacaine, lidocaine, and prilocaine. Interestingly, procaine and mepivacaine, which possess easily measureable anesthetic activity when administered by injection, provide only minimal surface anesthesia when applied topically. The precise reason for the poor topical anesthetic properties of these two agents has not been completely elucidated.

Onset and Duration of Action

It is difficult to differentiate between the onset time of various agents in animal models. Usually, onset of anesthesia occurs quite rapidly when the various compounds are employed in equieffective concentrations. Differences in onset time can be determined when epidural anesthesia is performed in animals such as cats, dogs, or sheep. Duce and co-workers demonstrated in cats that the onset of epidural anesthesia with lidocaine is decreased with an increased dose.[120] Addition of epinephrine and alteration in the pH of the local

anesthetic solution produced inconsistent changes in latency. Equieffective concentrations of lidocaine and tetracaine showed similar onset times.

The duration of motor block can be determined quite accurately in animals. On the basis of various types of peripheral or central neural blocks in different animal species, local anesthetic agents can be categorized as compounds of short, moderate, long, or ultralong duration of action. Procaine and chloroprocaine represent drugs of short duration of action both for peripheral nerve blockade and central neural blockade in animals. Lidocaine, mepivacaine, and prilocaine possess a moderate duration of action while tetracaine, bupivacaine, and etidocaine can be classified as agents of long duration of action. Tetrodotoxin and saxitoxin demonstrate ultralong anesthetic activity (Table 10).

Table 10

DURATION OF SPINAL ANESTHESIA IN SHEEP OF LIDOCAINE (50mg), TETRACAINE (10mg) AND TETRODOTOXIN (4 μg).

	LIDOCAINE	TETRACAINE	TETRODOTOXIN
DIGITAL PAIN	36 - 42 min	60 - 140 min	17 - 23 hrs
WEIGHT SUPPORT	38 - 60 min	165 - 202 min	17 - 23 hrs
FULL RECOVERY	60 - 85 min	282 - 405 min	25 - 28 hrs

n=4

The duration of local analgesia can be altered by factors such as the dosage of the agent and the addition of a vasoconstrictor substance, e.g., epinephrine, to the local anesthetic solution. Figure 3-6 depicts the dose-duration response curve for several agents employed for rat sciatic nerve blocks and epidural anesthesia in cats.[12, 120] An increase in duration of action occurs as the dosage is increased until a maximum value is reached or systemic toxicity occurs. The prolongation of analgesic activity bears a closer relationship to the total dosage than to concentration or volume of solution employed (Table 11).

Depending on the site of administration, vasoconstrictor agents can markedly increase the frequency of successful nerve blocks and prolong the duration of action of local anesthetic agents (Table 12).[88, 120, 125] The mechanism of this increased frequency and duration of anesthesia is related to a localized vasoconstriction in the area of administration, which results in a decreased systemic absorption of

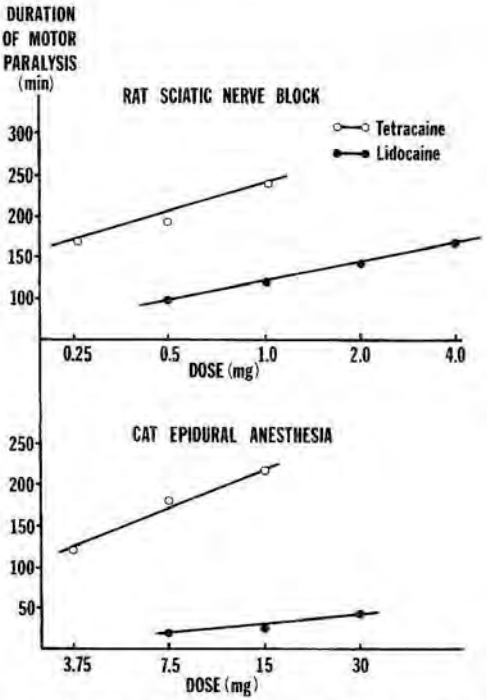

Fig. 3-6. Dose-duration relationship of tetracaine and lidocaine following rat sciatic nerve block and cat epidural anesthesia.

Table 11

ONSET AND DURATION OF HIND LIMB PARALYSIS IN CATS FOLLOWING EPIDURAL ADMINISTRATION OF VARYING VOLUMES, CONCENTRATIONS AND DOSAGE OF LIDOCAINE SOLUTIONS.

DOSE (mg)	15	15	30	30
CONCENTRATION (%)	1.0	1.5	1.5	2.0
VOLUME (ml)	1.5	1.0	2.0	1.5
ONSET (min) Mean ± S.D.	4.1 ± 0.9	5.9 ± 0.9	3.0 ± 0.9	2.4 ± 0.5
DURATION (min) Mean ± S.D.	22.1 ± 3.7	22.7 ± 5.7	35.7 ± 7.3	43.6 ± 5.6

Table 12

EFFECT OF EPINEPHRINE ON THE FREQUENCY AND DURATION OF PERIPHERAL AND CENTRAL NEURAL BLOCKADE

AGENT	ANESTHETIC PROCEDURE	WITHOUT EPINEPHRINE		WITH EPINEPHRINE	
		FREQUENCY OF COMPLETE BLOCK	DURATION OF ANESTHESIA (min)	FREQUENCY OF COMPLETE BLOCK	DURATION OF ANESTHESIA (min)
0.5% LIDOCAINE	CAT EPIDURAL ANESTHESIA	31.8%	20.4 ± 1.0	100%	32 ± 7.8
0.125% LIDOCAINE + TETRODOTOXIN 2 μg/ml	RAT SCIATIC NERVE BLOCK	20%	174	100%	368 ± 24

the anesthetic compound such that more of the agent is available for diffusion into neural tissue. Epinephrine is the agent usually added to local anesthetic solutions for the purpose of providing a localized state of vasoconstriction. Other sympathomimetic vasoconstrictor agents such as norepinephrine and phenylephrine also have been utilized to improve the frequency and duration of regional anesthesia (Table 13).[125] Nonsympathomimetic amine agents such as octapressin have also been studied as vasoconstrictor supplements for local anesthetic agents. Animal studies by Åkerman indicate that 0.9 μg/ml of octapressin may be equivalent to 3.33–5.0 μg/ml of epinephrine in terms of its ability to retard the absorption and prolong the anesthetic activity of lidocaine and prilocaine.[126]

Onset and duration of action can be altered by combining agents of rapid onset and short or moderate duration with substances possessing slow onset, but long or ultralong activity. Combinations of procaine or lidocaine with tetracaine or tetrodotoxin have been studied in animals, and the results suggest that the anesthetic mixture

Table 13

EFFECT OF VARIOUS VASOCONSTRICTOR AGENTS ON THE DURATION OF RAT SCIATIC NERVE BLOCKS PRODUCED BY 0.125% LIDOCAINE + 2μg/ml TETRODOTOXIN.

VASOCONSTRICTOR AGENT	CONCENTRATION	FREQUENCY OF COMPLETE BLOCK	DURATION (min)
NONE	—	20%	174
EPINEPHRINE	1:200,000	100%	368 ± 24
NOREPINEPHRINE	1:20,000	100%	354 ± 12
PHENYLEPHRINE	1:20,000	100%	377 ± 27

Table 14

EFFECT OF LOCAL ANESTHETIC MIXTURES ON ONSET TIME AND DURATION OF EPIDURAL ANESTHESIA IN DOGS

AGENTS	ONSET TIME (min)	DURATION (min)
1.0% LIDOCAINE	2.02 ± 0.33	123.8 ± 6.8
0.2% TETRACAINE	4.0 ± 0.66	272.4 ± 27.4
1.0% LIDOCAINE + 0.2% TETRACAINE	2.0 ± 0.20	282.7 ± 23.1

will possess the most favorable properties of the individual agents, i.e., rapid onset and long duration (Table 14).[125, 127] More detailed information concerning the relative onset and duration of local analgesic agents and the various factors which may influence their pharmacological properties can be obtained from studies in man (Chapter 4).

SUMMARY

1. The intrinsic anesthetic potency of a chemical compound is usually defined as the minimum concentration required to produce within 5–10 minutes a 50% reduction in the amplitude of the surface action potential recorded from an isolated nerve preparation.
2. Intrinsic anesthetic potency (Cm) may be influenced in vitro by various factors such as frequency and intensity of stimulation, type of isolated nerve preparation, presence or absence of a neural sheath and pH of anesthetic solution.
3. The clinically useful agents can be categorized as compounds of (a) low potency, e.g., procaine; (b) moderate potency, e.g., mepivacaine, prilocaine, chloroprocaine, and lidocaine; and (c) high potency, e.g., tetracaine, bupivacaine, and etidocaine.
4. Onset and duration of conduction blockade are related to pK_a, lipid solubility, and protein binding. In general, a lower pK_a and higher lipid solubility are associated with a more rapid onset time, while a higher protein-binding capacity is related to a longer duration of action.
5. The relative in vivo anesthetic potency, onset, and duration of different agents are similar to, but not identical with, in vitro

results. The observed differences betweeen in vitro and in vivo properties are due to other pharmacological actions of local analgesic drugs, such as vasodilation.

6. On the basis of in vivo animal studies, the various agents may be classified according to their relative durations of action, i.e., (a) short duration, e.g., procaine and chloroprocaine; (b) moderate duration, e.g., lidocaine, prilocaine, and mepivacaine; (c) long duration, e.g., tetracaine, bupivacaine, and etidocaine; and (d) ultralong duration, e.g., tetrodotoxin and saxitoxin.