In Vivo Evaluation of Local Anesthetics Applied Topically

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A method has been offered for the screening of topically applied anesthetic agents of varying molecular structure. It is based on the observation that injuring, but not necessarily rupturing the skin, reduces its resistance to penetration.

THE ADMINISTRATION of chemical agents through the skin has long been the object of considerable investigation which, in isolated instances, has proved fruitful; but generally has been frustrated by as yet undefined or unproved explanations of normal skin function.

Among the types of compounds with which there has been limited success (1) has been the agent which reduces discomfort resulting from relatively minor accessible injuries—the local anesthetic.

A variety of topical anesthetic testing methods are contained in the literature. These have included studies in the lingual receptors of the frog (2), the nasal and bucco-pharyngeal mucosae of the rabbit (3, 4), the tail of the earthworm (5), the goldfish (6), the eyes of cats, dogs, guinea pigs, and mice (7–10), as well as a host of *in vitro* techniques.

No method could be found by which the potency of an anesthetic agent could be determined, following its application to the surface of the skin of the more commonly used laboratory animals. In an effort to fill this need, a series of testing procedures was developed. They have been successfully employed in these laboratories for several years. They are based on the observation that superficial injuries which need not necessarily rupture the skin, may nonetheless alter the natural dermal barrier and thereby permit penetration of local anesthetic molecules.

METHODS

Each of the studies presented herein conforms to a basic six-step pattern: (*a*) determination of preinjury skin sensitivity; (*b*) the infliction of the injury; (*c*) the determination of postinjury, or pretherapy, skin sensitivity; (*d*) application of the agent; (*e*) determination of posttherapy sensitivity; and (*f*) evaluation of the results.

Twenty-four hours before the study, the backs of the guinea pigs were shaved with mechanical clippers and depilated with a commercial depilatory.[1]

During the test, no more than two animals were used at one time by one investigator. This rule tended to minimize the time gap between infliction of the injury and application of the agent. In addition, the simultaneous handling of but two animals permitted adequate opportunity for observation of sometimes subtle, but nonetheless discernible, reactions.

Because there is no other way of determining whether pain has been abolished, in the treated tissue of the laboratory animal, a stimulus must be applied to the test site and an absence of response is interpreted as being indicative of a state of anesthesia. Thus, even though almost any type of pain-inducing stimulation may be used, it is important that the nature, duration, intensity, and frequency be sufficiently controllable as to make the stimulus reproducible.

The manner in which each guinea pig reacted to pain varied and because of this, its individual type of response had to be established before the experimental agents were applied. Only the more demonstrable animals, which reacted in unmistakable fashion to the stimuli, were used.

The type of stimulus selected was relatively superficial, from the point of view of penetration; but it was of constant duration and was also controllable. It consisted of pricking the test area on the back of the immobilized guinea pig with the point of a 22-gauge hypodermic needle which was attached to the plunger of a 10-ml. plastic disposable syringe. The rubber tip of the plunger had been first removed to facilitate its free movement within the barrel. The barrel of the syringe was attached to a ring stand in a vertical position at a constant height. The distance from which the plunger (with the attached needle) was dropped before striking the test site on the guinea pig's back, as well as the addition or subtraction of weights taped to the top of the plunger, determined the intensity of the stimulus applied. Furthermore, by dropping the plunger from the same height (as determined by the calibrations on the barrel of the syringe) a stimulus could be duplicated.

The sensitivity levels were determined by applying stimuli, the intensities of which were only of sufficient magnitude to have elicited consistent and reliable responses, and a state of anesthesia was considered to have been induced when the previously established postinjury sensitivity had been abolished following administration of the experimental agent.

In order to determine whether any specific area of the denuded back may have been more suitable, preliminary studies were run on varying sections.

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It soon became apparent that the skin directly over the scapulae was more sensitive and yielded higher levels of consistency. Thereafter, those areas were used exclusively.

Two test sites were therefore used on the back of each animal (one over each scapula) for all but the Type II burn study.

The Type I burn was made by momentarily touching the skin with the broad side of a 2.21-cm. (7/8 in.) steel spatula. Heating was effected by holding the spatula above the 3- to 5-mm. high blue portion of a flame emitted from a Fisher Wide Flame gas burner for 15 sec.

The Type II burn (the scald) was inflicted by immersing the test area in a 80° bath for 5 sec. Here, as has been mentioned, only a single burn was made on the back of each animal.

Type III burns were caused by immobilizing the animals beneath a 250-w. Lo-Glare ceramic IR lamp for periods of 5 min. at a distance of 21.59 cm. (8 1/2 in.). Windowed shields of aluminum foil attached to the backs, restricted the areas of exposure to approximately 1 sq. in. at each of the two sites.

The experimental preparations were also tested on an abrasion which was induced by scraping the skin (5 strokes in one direction; then 5 more strokes at a right angle) with 150 (2/0) garnet carborundum paper.

As was mentioned above, the sensitivity (threshold) was again established after each injury and it was consistently noted that a stimulus of much less intensity was required to cause reactions after inflictions of the injuries.

Of significant importance was the observation that all portions of the skin, damaged by Type I burns, were equally sensitive to stimuli of constant intensity. This was not the case, however, following the infliction of Type II burns. The periphery of the scalded areas seemed very sensitive and lightly applied pricks elicited markedly exaggerated protests from the guinea pigs. As points nearer the center of the burn were stimulated, reactions tended to diminish and, in the center of the burn, levels of response which were inferior to those detected prior to burning, were noted. Whether this apparent inhibition of impulse conduction was due to damage of the superficial nerve fibers, or was due to a dampening effect created by the presence of edema fluid, was not established. Because of this variation, the application of all stimuli was restricted to a zone approximately 6 mm. in width, around the circumference of the scald...even though the experimental compounds were applied to the entire burned area. This zone was the most sensitive and yielded the most constant results. As was true following the Type I burns, those areas which had been injured by Type III burns, as well as abrasions, each exhibited increased sensitivity and all portions of the injured areas reacted equally to constant stimuli.

To illustrate the method, three known anesthetic agents diluted in a 80:20 propylene glycol–water mixture were applied at two concentrations, 0.125 and 1.000% (w/v). These agents were lidocaine-(diethylaminoacet-2, 6-xylidide); benzocaine(ethyl aminobenzoate); and diperodon[3-(1-piperidyl)-1,2-propanediol dicarbanilate].

Following the administration of each test preparation, stimuli equivalent to those required to have caused acceptable postinjury responses were applied at 1-min. intervals until a total of 10 had been made. Following this, they were again stimulated 15 to 20 min. after the application.

Every preparation was tested on a minimum of six sites to which the same type of injury had been inflicted; but on no occasion was the same agent applied to more than one site on the same animal.

Finally, in addition to being subjected to the procedures described, it was felt that efficacy, following application to optic tissue, should be considered when screening for topical anesthetic potency. Toward this end, the preparations were tested in the rabbit and guinea pig *via* the method of Chance and Lobstein (9). The validity of their procedure, as well as that of the newly proposed method, depends on acceptance of the above-stated premise that absence of response in a treated site, following application of a normally effective stimulus, is indicative of a state of anesthesia. In the eye, the relative potencies of topically administered agents were determined on the basis of responses to corneal stimuli. These, in the form of gentle strokes on the cornea with a single camel hair, were applied once each min. for the next 18 consecutive min. following treatment.

RESULTS

The number of "no responses" to stimuli applied to each test area have been presented individually in Table I. As recommended by Chance and Lobstein, optic sites were each stimulated 18 times; whereas only 12 stimuli were applied to injured skin sites.

The number of negative responses for each test group of six were totalled and then divided by the number of stimuli—thereby yielding single values (see Table II) which represented the anesthetic potencies of the preparations in the respective sites. A score of 1.000 was indicative of an onset of action of less than a min. and a duration of at least 18 min. in the eye or 20 min. in the skin. Values of lesser magnitude were proportional to the degree of anesthesia elicited. Also presented in Table II are the fiduciary limits for each value, calculated with a probability limit of 95%. Comparisons of these supported the following conclusions.

Rabbit Eye—(*a*) The 0.125% concentrations of none of the three test agents was significantly more potent than the water control. (*b*) Diperodon (1.0%) was more potent than 1.0% lidocaine. (*c*) Lidocaine (1.0%) was more potent than 1.0% benzocaine.

Guinea Pig Eye—(*a*) As compared with the water control, all six experimental solutions were significantly more active. (*b*) None of the three agents, when applied at concentrations of 0.125%, caused significantly different levels of response. (*c*) Lidocaine (1.0%) was more potent than 1.0% benzocaine. (*d*) Diperodon (1.0%), with a potency value which was between those of lidocaine and benzocaine, proved to have been no less potent than 1.0% lidocaine but simultaneously, no more potent than 1.0% benzocaine. The confidence limits of the diperodon value overlapped the confidence limits of both of the other solution values.

Abraded Skin—(*a*) All solutions, except the

TABLE I—TOTAL NUMBERS OF NEGATIVE RESPONSES OBSERVED AT EACH OF THE SIX SITES WHICH WERE EMPLOYED PER COMPOUND PER ANALYSIS

Compd.	Rabbit Eye	Guinea Pig				
		Eye	Abraded Skin	Burns Type I	Burns Type II	Burns Type III
0.125% benzocaine	0,0,1,0, 0,0	0,2,3,1, 4,2	0,0,0,0, 0,0	2,2,0,1, 0,0	0,0,0,0, 0,0	2,3,2,0, 1,2
0.125% diperodon	0,1,1,0, 0,0	2,0,1,3, 1,2	3,4,4,3, 5,3	3,0,2,2, 1,2	0,0,0,0, 0,0	0,0,0,0, 0,0
0.125% lidocaine	0,0,0,0, 0,0	0,2,4,1, 1,2	2,2,0,3, 1,1	0,0,2,1, 0,0	0,0,0,0, 0,0	0,0,0,0, 0,0
1.000% benzocaine	3,2,2,0, 2,3	7,4,6,6, 5,6	4,2,5,3, 2,4	2,1,2,1, 3,2	5,4,5,6, 4,6	5,4,4,4, 3,3
1.000% diperodon	9,12,16, 15,12,14	7,10,8 8,11,9	6,7,6,4, 5,5	4,1,2,2, 4,3	1,2,2,0, 1,0	0,0,0,0, 0,0
1.000% lidocaine	8,5,7,7, 8,9	10,11,12, 15,14,9	2,1,3,3, 3,2	1,2,0,0, 3,0	2,4,2,3, 1,2	0,0,0,0, 0,0
Water control	0,0,0,0, 0,0	0,0,0,0, 0,0	0,0,0,0, 0,0	0,0,0,0, 0,0	0,0,0,0, 0,0	0,0,0,0, 0,0

0.125% benzocaine, were significantly more active than the water control. (*b*) Diperodon (0.125%) was more potent than 0.125% lidocaine. (*c*) Diperodon (1.0%) was more potent than 1.0% lidocaine. (*d*) Benzocaine (1.0%), with an intermediate value, was no less active than 1.0% diperodon, but no more active than 1.0% lidocaine.

Type I Burn—(*a*) Of the six solutions tested, only 0.125% lidocaine exhibited no more potency than the water control. (*b*) There was no significant difference between the potency of 0.125% benzocaine and that of 0.125% diperodon. (*c*) There were no significant differences between the potencies of 1.0% diperodon, 1.0% lidocaine, and 1.0% benzocaine.

Type II Burn—(*a*) None of the three agents examined, when applied at concentrations of 0.125%, were more potent than the water control. (*b*) Benzocaine (1.0%) was significantly more potent than both 1.0% diperodon and 1.0% lidocaine. (*c*) The difference between the potency values of 1.0% diperodon and 1.0% lidocaine was not significant.

Type III Burn—(*a*) Of the three agents tested, only benzocaine, at both concentrations employed, proved to have been measurably effective in this type of injury. These findings have been subsequently confirmed in different vehicles as well as in blindly run studies.

From these data it seemed apparent that no single agent was consistently the local anesthetic of preference for all situations. Of those examined, diperodon may be the most effective in the eye and in the abraded skin—while any of the three may be correctly chosen to abolish pain in the Type I burn. Benzocaine, on the other hand, seemed most effective in the Type II and Type III burns. It was interesting to have noted that Adriani, following studies in the human, also found (11) that anesthetic agents which exhibited considerable potency after topical application to one type of site are not necessarily as active in another site.

DISCUSSION

Attention is directed to the quality of the data presented in Table I. An examination of these had led to the conclusion that (*a*) these are valid observa-

TABLE II—ANESTHETIC POTENCY SCORES (TOTAL "NO RESPONSES" DIVIDED BY THE TOTAL NUMBER OF APPLIED STIMULI PER COMPOUND PER ANALYSIS) AND THEIR RESPECTIVE 95% FIDUCIARY LIMITS

Compd.	Rabbit Eye	Guinea Pig				
		Eye	Abraded Skin	Burns Type I	Burns Type II	Burns Type III
0.125% benzocaine	0.009 (−0.009 to 0.027)	0.111 (0.052 to 0.170)	0.000	0.069 (0.011 to 0.128)	0.000	0.139 (0.059 to 0.219)
0.125% diperodon	0.018 (−0.007 to 0.044)	0.083 (0.031 to 0.135)	0.306 (0.200 to 0.412)	0.139 (0.059 to 0.219)	0.0000	0.0000
0.125% lidocaine	0.000	0.093 (0.038 to 0.147)	0.125 (0.049 to 0.201)	0.042 (−0.004 to 0.088)	0.000	0.000
1.000% benzocaine	0.111 (0.052 to 0.170)	0.315 (0.227 to 0.402)	0.278 (0.174 to 0.318)	0.153 (0.070 to 0.236)	0.417 (0.303 to 0.531)	0.319 (0.212 to 0.427)
1.000% diperodon	0.722 (0.638 to 0.807)	0.491 (0.396 to 0.585)	0.458 (0.343 to 0.573)	0.222 (0.126 to 0.318)	0.083 (0.019 to 0.147)	0.000
1.000% lidocaine	0.407 (0.315 to 0.500)	0.657 (0.568 to 0.747)	0.194 (0.103 to 0.286)	0.083 (0.019 to 0.147)	0.194 (0.103 to 0.286)	0.000
Water control	0.000	0.000	0.000	0.000	0.000	0.000

TABLE III—DATA ILLUSTRATING LEVELS OF CONSISTENCY FOLLOWING REPEATED TESTING OF 1.0% LIDOCAINE IN DERMAL INJURIES

Experiment No.	Skin Abrasion		Type I Burn		Type II Burn		Type III Burn	
	Individual Scores	Potency Value	Individual Scores	Potency Value	Individual Scores	Potency Value	Individual Scores	Potency Value
1	2,1,3,3,2,0	0.153	0,2,1,3,1,1	0.111	1,3,2,2,0,1	0.125	0,0,0,0,0,0	0.000
2	3,3,0,3,1,2	0.166	0,0,1,0,2,0	0.042	4,0,2,2,4,2	0.194	0,0,0,0,0,0	0.000
3	1,5,4,2,1,3	0.222	1,1,0,2,0,0	0.056	3,1,0,2,4,1	0.153	0,0,0,0,0,0	0.000

tions and not the result of chance, and (*b*) the values within groups have consistent variance and therefore are considered compatible.

Of course the potency scores in Table II, which are derived from the data in Table I, differ significantly only when the confidence limits do not overlap.

The importance of reproducibility cannot be too strongly emphasized. An example of the level of consistency which may be expected by use of this procedure has been presented in Table III. Contained therein are the results of repetitive studies of the above described 1.0% lidocaine solution. These results, it will be noted, neither differ significantly from each other nor from those in Tables I and II. Consistency of approximately the same order has been yielded by repeated topical studies of other experimental compounds.

The breadths of the confidence limits, which embraced the respective potency scores, were of concern. It was found, however, that these ranges could be narrowed considerably by merely supplementing the numbers of sites employed. After consideration, it was decided that the initial use of but six sites was sufficient to provide results which satisfied requirements of an initial screen; and that the strengthening process of adding data should probably best be limited to accurate appraisal of the more promising compounds.

With regard to the mechanics of the method, it may be felt that receptors and nerve fibers may have been sufficiently damaged by the burns to possibly inhibit the transmission of impulses. If this were to occur, it may be argued that applied stimuli may fail to elicit responses and that erroneous results as to the potency of the experimental compounds may be yielded.

The fact that measurable decreases in the threshold responses can be demonstrated after injuring the skin certainly refutes this argument. Tissues within and beneath the dermal layers are highly sensitive to the painful influences of a histamine-like H-substance (12) which is liberated following infliction of skin injuries. With increased tissue damage, the source of the pain lies further from the surface and the challenge to the penetrating ability of the anesthetic agent is correspondingly increased. It should furthermore be remembered that the same injury which causes the pain also alters the permeability, thereby providing the means by which the anesthetic may be permitted to penetrate.

It should again be emphasized that it is not the intent here to become involved in such matters as structure-activity relationships, penetration of the dermal barrier, biochemical or pathologic changes in skin following thermal injury, or any of a countless number of other related problems of a fundamental nature.

Instead, a method has been offered which, despite acknowledged minor fluctuations in response, due to variation in the animals as well as slight differences in the severities of the injuries, has repeatedly proven to have been accurate and reproducible; and has permitted the simultaneous evaluation of the spectra of activity of structurally different anesthetic agents.

CONCLUSION

A series of procedures for the *in vivo* testing of topical anesthetic agents on the superficially injured skin of the rodent has been presented. The relative potencies of three known agents were evaluated and the results indicated that (*a*) diperodon was the anesthetic of choice in nullifying pain in the abrasion; (*b*) lidocaine, benzocaine, and diperodon were equally effective in one type of burn; and (*c*) benzocaine exhibited superior activity in two additional types of burns.

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Keyphrases

Anesthetics, topical—*in vivo* evaluation
Burns, controlled infliction—guinea pig back
Abrasions, inflicted—guinea pig back
Eyes, rabbit, guinea pig—anesthesia testing
Stimuli application—anesthesia determination