Pharmacology Biochemistry & Behavior, Vol. 14, pp. 67–73. Printed in the U.S.A.

Infusions into the Oculomotor Nucleus or Nerve: A Method of Estimating the Dosage at which Transmitter Antagonists Infused Intracranially Produce Nonspecific Blocking of Neural Activity[1]

D. J. ALBERT

Psychology Department, University of British Columbia, Vancouver, B.C., Canada

Received 29 May 1980

ALBERT, D. J. *Infusions into the oculomotor nucleus or nerve: A method of estimating the dosage at which transmitter antagonists infused intracranially produce nonspecific blocking of neural activity.* PHARMAC. BIOCHEM. BEHAV. 14(1) 67–73, 1981.—Lidocaine, a local anesthetic, was first infused into the oculomotor nucleus or nerve in anesthetized rats through one barrel of a two barrel infusion needle and the extent of pupillary dilation measured. Ten to fifteen minutes after the pupillary diameter had returned to its preinfusion level, a transmitter antagonist was injected through the second barrel of the cannula and its effect on pupillary diameter measured. Lidocaine, when in close proximity to the oculomotor nucleus or nerve produced a dilation of 3 to 4 mm while saline produced no consistent change. Infusion of phentolamine (α-adrenergic), tolazoline (α-adrenergic), atropine (cholinergic), and haloperidol (dopaminergic) but not propranolol (β-adrenergic) produced increases in pupillary diameter as large as those produced by lidocaine whether infused along the nerve or into the nucleus. In a second experiment using unanesthetized rats, dilation by each of these substances and atropine methyl nitrate and propranolol induced dilation when infused into the oculomotor nucleus. In a final experiment, infusion of glutamate and aspartate but not cholinergic (carbachol), noradrenergic (norepinephrine), dopaminergic (dopamine) agonists produced pupillary constriction. These results suggest that pupillary control by the oculomotor nucleus is sensitive to glutamate and aspartate but not to several other well known transmitter substances. It, therefore, can serve as a useful site for the evaluation of the nonspecific suppression of neural activity caused by various transmitter antagonists as well as a variety of other excitatory and inhibitory substances.

Atropine Haloperidol Intracranial injection Oculomotor nucleus Phentolamine Propranolol Tolazoline

THE infusion of drugs into specific brain regions is frequently used to identify the transmitter substance associated with neural activity controlling a particular behavior. Many of these agents produce a nonspecific suppression of neural activity when present in the higher concentrations that are frequently used for intracranial injections [9, 12, 30, 31]. This nonspecific suppression may be the result of a local anesthetic or a nonspecific transmitter blocking effect. Some kind of nonspecific suppression of neural activity was indicated when we recently discovered that the hyperreactivity and mouse killing induced by intracranial infusion of the α-adrenergic antagonist tolazoline was not abolished by prior destruction of noradrenergic neurons at that site by the neurotoxin 6-hydroxydopamine [4].

The present report describes a method which can be used to estimate the dosage at which nonspecific suppression effects of intracranially infused transmitter antagonists appear. The method is to induce the agent of interest into either the oculomotor nucleus or the efferent oculomotor nerve and then observe the change in pupillary dilation. A suppression of activity in this nucleus or its efferent nerve will result in pupillary dilation [1]. An enhancement of neural activity in the nucleus or nerve will result in pupillary constriction [32].

EXPERIMENT 1: INFUSION OF TRANSMITTER ANTAGONISTS INTO THE OCULOMOTOR NUCLEUS OR NERVE IN ACUTE PREPARATIONS

Infusions into the oculomotor nucleus or nerve were made under acute conditions using a double-barreled can-

[1]Supported by a grant from the National Research Council of Canada. The author is indebted to Drs. G. Davis, H. McLennan, C. Broekkamp, F. Madryga, and to K. N. Brayley for helpful discussions. The authors are indebted to Ayerst Laboratories, Ciba Canada, Allen and Hanburys, and McNeil Laboratories for generous supplies of drugs. Reprint requests: D. J. Albert, Psychology Department, University of British Columbia, Vancouver, B.C., Canada V6T 1W5.

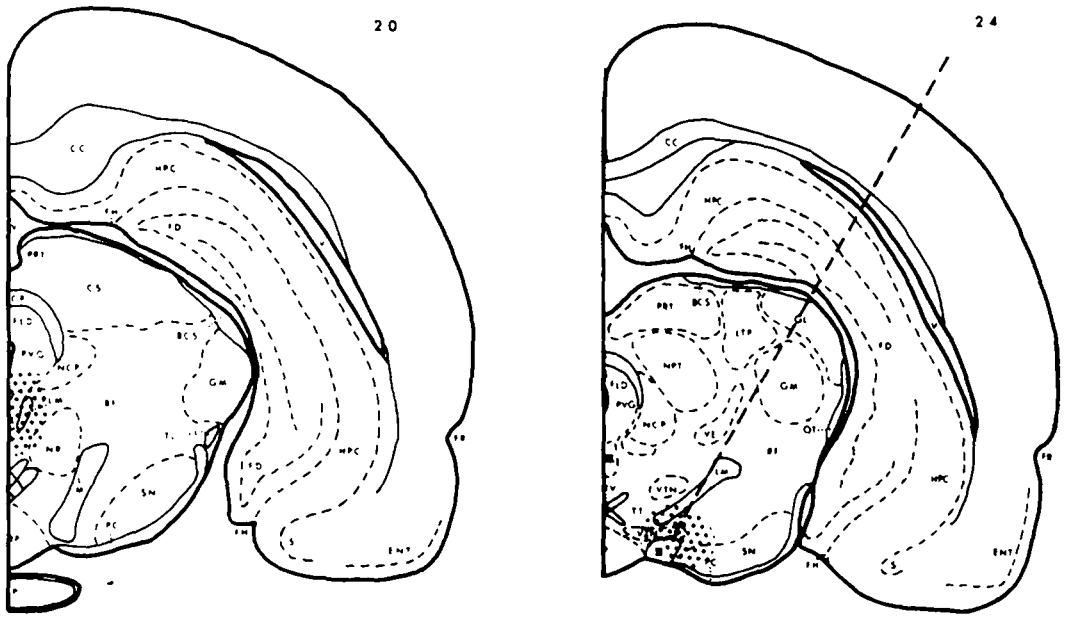

FIG. 1. The location of the injection sites into the oculomotor nucleus (left, shaded area) and the oculomotor efferent nerve (right, shaded area). The track of the infusion needle in its approach to the oculomotor nerve is also shown (dashed line). Drawings are from Pellegrino and Cushman [23]

nula. A local anesthetic (lidocaine) was first infused from one barrel of the injection needle. If the cannula was in close proximity to the nucleus or the nerve, pupillary diameter increased by up to four millimeters. When the dilation had dissipated, a transmitter antagonist was infused from the second barrel and its effect on pupillary diameter recorded. With each transmitter antagonists at least one experiment was done with the specific antagonist injected first to ensure that the blocking effects obtained were not due to prior injection of lidocaine.

METHOD

The experiments were performed on male hooded rats (350–450 g) which were anesthetized with sodium pentobarbital (50 mg/kg) or urethane (1.25 g/kg). Supplemental doses of sodium pentobarbital were given when the corneal reflex became active, although never during or immediately after intracranial infusion of any drugs. All drugs were evaluated under both urethane and sodium pentobarbital anesthesia.

The animals were held in a Kopf stereotaxic instrument. The double-barreled infusion needle which was inserted into the brain stereotaxically consisted of two pieces of 30 g stainless steel hypodermic tubing soldered side by side. The tips were beveled at about 45°C so that the orifices of the two cannulas were at the same level and faced in the same direction. Each barrel of the infusion needle was connected by polyethylene tubing (P.E. 50, Intramedic) to a 50 μl syringe (Hamilton). Each syringe was held in a Sage Instrument pump (Model 341) which controlled the rate of infusion.

Measurement of Pupillary Dilation

Pupillary diameter was measured with the aid of a Zeiss binocular microscope with a through lens light beam and times six magnification. The measuring device was a small flexible plastic ruler which could be held next to the cornea. The ruler was graduated in 1 mm divisions and measurements were estimated to the nearest 0.25 mm.

Drug Infusion Sites

The infusion sites are shown in Fig. 1. The barrels of the infusion needle were aligned in the sagittal plane for injections into the nucleus and in the coronal plane with injections into the nerve.

Drug Testing Procedure

After the infusion needle was lowered into the brain, the pupillary diameter in each eye was measured every 5 min until the infusions began. With infusions along the descending nerve, the infusion began 10 min after the needle was lowered. A slightly longer delay was allowed before injections into the nucleus (15 min) since it was throught that the cell body region might be more sensitive to the mechanical disturbance caused by the needle.

The routine procedure was to first infuse 2μl of lidocaine (Xylocaine, 2%, Astra), a general blocking agent, to determine whether the tips of the infusion needles were at the desired site. The infusion rate was 1 μl per 3 min. A one min interval was left between the infusion of each microliter to allow for measurement of pupillary diameter unconfounded by any mechanical disturbance due to the infusion itself. A second measurement was made after the second microliter was infused. Additional measurements were made at 5 min intervals until the pupil returned to its preinfusion baseline

level. The order in which the pupils were measured was alternated. If the lidocaine produced an increase in pupillary dilation of 2.0 mm or greater in at least one eye the animal was retained in the experiment.

After the pupillary diameter returned to the preinjection level, a further 10 min was allowed before the infusion of the test substance began. Infusion of 2 μl of the test substance (a transmitter antagonist) was made through the second barrel of the injection needle without altering the needle's placement. The infusion procedure was the same as with licocaine. Following infusion of the test transmitter antagonist, pupillary measurements were again made at 5 min intervals until the pupillary diameter returned to baseline level.

With infusions into the oculomotor nucleus, only one pair of injections was made into each animal. Both of the descending efferent nerves, however, were sometimes used for a separate test.

The drugs used in the present experiments were tolazoline hydrochloride (Priscoline, Ciba), phentolamine mesylate (Rogitine, Ciba), propranolol hydrochloride (Inderal, Ayerst), atropine sulfate (Atropine, Allen and Hanburys) and haloperidol (Haldol, McNeil). Phentolamine, propranolol, and atropine sulfate were dissolved in distilled water. The main experiments with tolazoline were done using the commercially available preparations, but the effects were the same when injected in saline solutions. Haloperidol was dissolved in 0.3% tartaric acid.

Histology

At the termination of the experiment each animal was killed by cervical dislocation. The brain was placed in formol-saline, sectioned and stained with cresyl violet. The infusion sites from which dilation was produced were all in close proximity to the nucleus or nerve [1].

RESULTS

Pupillary dilation caused by the infusion of phentolamine, tolazoline, atropine sulfate, or haloperidol into the oculomotor nucleus or the oculomotor nerve is shown in Tables 1 and 2. With infusions into the nucleus (Fig. 1) the dilation was always bilateral although not of equal extent. With infusions into the oculomotor efferent nerve (Fig. 1), the dilation was always unilateral to the side of the injection. The dilation shown in Tables 1 and 2 occurred with both urethane and sodium pentobarbital anesthesia. In addition, the dilation also occurred when the drug was infused prior to lidocaine. There was no change from baseline dilation with the infusion of propranolol (4,8, or 40 ug; N=7), or saline.

A control group was run for animals infused along the nerve in order to ascertain whether the dilation obtained with the transmitter antagonists was due to spread to the nucleus. In these animals, the drug was injected along the trajectory toward the nerve but at a point 2 mm short of the nerve itself. In no animals infused in this way was there a pupillary dilation.

Statistical tests were not done on the pupillary changes because the main drug effects reported involved dilations of 2 to 4 mm, were measured several times in each animal, and were far in excess of the minor effects seen with saline. Also, the results for each drug will be repeated in the following experiment in unanesthetized rats.

TABLE 1

MAXIMUM PUPILLARY DILATION FOLLOWING THE INFUSION OF VARIOUS AGENTS INTO THE OCULOMOTOR NUCLEUS IN ANESTHETIZED ANIMALS. THE PREINFUSION BASELINE WAS 0.5 mm OR LESS IN ALL ANIMALS

		Dilation in mm (± S.E.M.)	
Antagonist	N	Lidocaine	Antagonist
Tolazoline (12.5 μg)	3	+3.8 (±0.3)	+0.6 (±0.5)
Tolazoline (25 μg)	2	+2.9 (±1.1)	+2.3 (±1.3)
Tolazoline (50 μg)	4	+3.5 (±0.2)	+3.6 (±0.2)
Phentolamine (5 μg)	3	+2.3 (±0.3)	+0.4 (±0.4)
Phentolamine (10 μg)	4	+3.9 (±0.1)	+3.3 (±0.7)
Atropine Sulfate (4 μg)	2	+3.9 (±0.6)	+0.8 (±0.8)
Atropine Sulfate (10 μg)	4	+3.3 (±0.5)	+1.7 (±0.5)
Haloperidol (10 μg)	3	+3.7 (±0.2)	+3.5 (±0.3)
Propranolol (40 μg)	4	+3.6 (±0.2)	+0.3 (±0.3)
Saline (0.9%)	3	+3.5 (±0.4)	+0.2 (±0.2)

TABLE 2

MAXIMUM PUPILLARY DILATION (MM ± S.E.M.) PRODUCED BY THE INFUSION OF VARIOUS AGENTS ALONG THE OCULOMOTOR NERVE IN ANESTHETIZED RATS. THE PREINFUSION BASELINE WAS 0.5 mm OR LESS IN ALL ANIMALS

Antagonist	N	Lidocaine	Antagonist
Tolazoline (12.5 μg)	2	3.3 (±0.3)	0.0 (±0.0)
Tolazoline (25 μg)	2	3.4 (±0.1)	1.8 (±1.3)
Tolazoline (50 μg)	4	3.3 (±0.4)	3.0 (±0.6)
Phentolamine (5 μg)	2	3.1 (±0.1)	0.0 (±0.0)
Phentolamine (10 μg)	3	3.5 (±0.0)	2.5 (±0.6)
Atropine Sulfate (1.2 μg)	2	4.0 (±0.0)	0.0 (±0.0)
Atropine Sulfate (4 μg)	2	3.8 (±0.3)	2.5 (±0.5)
Atropine Sulfate (10 μg)	5	3.1 (±0.3)	2.6 (±0.4)
Haloperidol (5 μg)	2	3.0 (±0.0)	0.5 (±0.3)
Haloperidol (10 μg)	3	4.0 (±0.3)	2.9 (±0.7)
Saline (0.9%)	3	3.8 (±0.3)	0.3 (±0.2)

DISCUSSION

Infusion of lidocaine into the oculomotor nucleus or along the efferent nerve produced a large and readily measureable pupillary dilation that was stable, free of erratic changes, and of sufficient duration to allow successive measurements to confirm one another. The ineffectiveness of the saline infusions establish that fluid in the two barrels of the infusion needle do not substantially contaminate one another and that the mechanical action of the infusion does not itself produce a substantial dilation.

An important point for purposes of the present experiment is whether fluid injected along the nerve would spread to the nucleus. Two findings suggest that this does not occur. First, injection along the tract the injection needle follows in approaching the nerve did not cause dilation in spite of the fact that at the infusion site along the needle's trajectory toward the nerve, the distance away from the oculomotor nucleus was approximately the same as at the infusion site

along the nerve. Second, with infusions along the nerve, dilation was always unilateral. It seems virtually impossible that the drug could have spread 2 mm to the nucleus and then affected only one side of the nucleus.

The fact that the various transmitter antagonists blocked neural activity along the nerve as well as in the nucleus suggests that the suppression of neural activity was due to a local anesthetic effect. This suggestion has been made previously for atropine sulfate by Curtis and Phyllis [9] who also drew attention to the structural similarity of atropine and cocaine. An examination of the structures of phentolamine, tolazoline, propranolol, and haloperidol reveals that each of these also bears a structural resemblance to local anesthetics in that each consists of an aromatic residue, an intermediate carbon chain, and an amino group.

To further establish whether any of the transmitter antagonists were producing a nonspecific suppression of neural activity it will be necessary to determine whether the suppression also occurs in unanesthetized rats and whether the complementary agonist of each would cause constriction. On the basis of existing histochemical evidence, there is reason to believe that none of the effects were specific. Roffler-Tarlov and Tarlov [27] have found no indication of the synthesis of acetylcholine, noradrenaline, or dopamine in the oculomotor nucleus of the cat. The study of the catecholamine systems in the brain gives no indication that the pupillary control by the oculomotor nucleus is dopaminergic or noradrenergic [21,34].

EXPERIMENT 2: INFUSIONS OF TRANSMITTER ANTAGONISTS IN UNANESTHETIZED RATS

The use of anesthetized rats in the previous experiment raises the possibility that the nonspecific effects of the transmitter antagonists were augmented by the general anesthetic. This obviously was the case in the previous experiment because the dose of lidocaine necessary to obtain a maximal effect at a particular injection site was 2 rather than 4 μl as we have found previously [1, 2, 3]. Further, the dilation endured longer following the termination of the infusion than we have found with other behaviors [1, 2, 3, 4, 5].

The present experiment examined the dilation produced by various transmitter antagonists in unanesthetized animals in which infusion cannulas had previously been chronically implanted into the oculomotor nucleus.

METHOD

The subjects were from the same population as those in the previous experiment.

A single stainless steel cannula was implanted into the oculomotor nucleus using standard surgical procedures. The cannula was made from 23 g and the needles from 30 g stainless steel tubing.

Injections were made beginning a minimum of 4 days postoperatively. Each animal was subsequently infused on a maximum of 4 occasions with a minimum of 48 hr between each infusion. The infusion procedure was similar to that of the previous experiment except that the rat was only infused with one drug on any day. Also, each animal was infused with 4 rather than 2 μl. Pupillary dilation was measured in the manner described for the previous experiment.

Lidocaine was infused first in all animals. Subsequent drugs were infused randomly. The drugs infused were the same as in the previous experiment plus atropine methyl nitrate (Sigma).

Following the drug infusion the brains of all animals were removed, placed in formol-saline, sectioned, and stained with cresyl violet. The cannulas of all animals in which there was dilation were found to be in close proximity to the nucleus [1].

RESULTS AND DISCUSSION

Phentolamine, atropine sulfate, tolazoline, and haloperidol each induced dilation (Table 3). The effective doses were approximately twice those in the anesthetized preparation of Experiment 1 (Table 1). However, propranolol which was not effective in anesthetized rats was effective in unanesthetized animals. Atropine methyl nitrate which was not tested in the first experiment produced dilation at about half the dose as atropine sulfate. With each antagonist, the appearance of the dilation was somewhat proportional to the amount infused, so that some dilation occurred following one-quarter and one-half the dose. The duration of the dilation differed from one drug to another. With lidocaine, tolazoline, phentolamine, and haloperidol the dilation disappeared within 10 min of the end of the infusion. With propranolol the dilation lasted from 10 to 20 min while with atropine sulfate or atropine methyl nitrate the dilation took from 15 to 30 min to disappear. The baseline level of dilation in all animals was 0.25 to 0.5 mm.

These results support the inference that at some concentration a variety of transmitter antagonists will produce a nonspecific suppression of neural activity. Further, the effective dose is not exceptionally higher than the dose used in many behavioral experiments.

EXPERIMENT 3: INFUSION OF TRANSMITTER AGONISTS

To be sure that the blocking effects found in the previous experiments were not specific it is necessary to ascertain whether transmitter agonists will produce the complemetary behavioral change; in this case, pupillary constriction. Rats were implanted with cannulas into the oculomotor nucleus. The appropriateness of the cannula placement was first evaluated by determining whether infusion of lidocaine produced a pupillary dilation. On a subsequent test day, a transmitter agonist was injected while the animal's pupil was maintained in a partially dilated state by adaptation to low intensity red light.

METHOD

The animals and surgery were similar to those used in the second experiment.

After a minimum of 4 days postsurgical recovery, each rat was first infused with lidocaine to determine whether the cannula was close enough to the pupil to produce pupillary dilation. The infusion procedure was that used in Experiment II. Animals continued in the experiment only if the dilation produced by lidocaine was 2.0 mm or greater.

Subsequent infusions were made with transmitter agonists beginning a minimum of 2 days afterward. The animals were put for 20 min into a room which was dimly illuminated by red light. The infusion needles were then inserted. At two 5 min intervals thereafter, readings of pupillary dilation were made under the dim illumination with the aid of a plastic ruler and dim red light from a small flashlight. Five

TABLE 3

THE PUPILLARY DILATION CAUSED BY INFUSION OF VARIOUS TRANSMITTER ANTAGONISTS INTO THE OCULOMOTOR NUCLEUS. THE PREINFUSION BASELINE WAS 0.5 mm OR LESS IN ALL ANIMALS

		Maximum Dilation in mm (± S.E.M.)	
Antagonist	N	Lidocaine	Antagonist
Phentolamine (4 μg)	3	+2.5 (±0.3)	+0.8 (±0.4)
Phentolamine (20 μg)	5	+3.4 (±0.2)	+1.9 (±0.5)
Tolazoline (50 μg)	3	+4.0 (±0.0)	+1.2 (±1.0)
Tolazoline (100 μg)	5	+3.1 (±0.2)	+2.8 (±0.3)
Propranolol (4 μg)	2	+2.8 (±0.3)	+0.5 (±0.0)
Propranolol (20 μg)	5	+2.8 (±0.3)	+2.7 (±0.3)
Propranolol (40 μg)	2	+4.0 (±0.1)	+2.9 (±0.4)
Methyl Atropine (4 μg)	2	+3.3 (±1.1)	+0.8 (±0.4)
Methyl Atropine (10 μg)	2	+4.0 (±0.0)	+2.4 (±2.3)
Methyl Atropine (20 μg)	5	+3.7 (±0.2)	+3.8 (±0.1)
Atropine Sulfate (4 μg)	3	+3.2 (±0.5)	+0.1 (±0.1)
Atropine Sulfate (20 μg)	11	+3.0 (±0.1)	+2.0 (±0.1)
Haloperidol (8 (μg)	2	+2.2 (±0.4)	+1.0 (±1.2)
Haloperidol (20 μg)	4	+3.0 (±0.3)	+3.0 (±0.3)
Saline (0.9%)	5	+2.8 (±0.7)	+0.5 (±0.3)

minutes after the second baseline level of dilation was measured, the infusion process began.

On the basis of preliminary exploratory experiments with various transmitter agonists, a slightly different infusion procedure was devised for use with the agonists. This procedure was used in order to minimize the amount of agonist used to produce dilation and to use an amount that would result in a relatively rapid return to normal dilation when the infusion stopped. With the procedure used the infusion rate was 1 μl/3 min as in the previous experiment, but instead of infusing continuously, the infusion pump was on only every other minute. Thus, one μl was infused over a 5 min period. A total of 2 μl was infused into each animal. Measurements of pupillary dilation were made after each μl and at 5 min intervals following the infusion until dilation reached its preinfusion baseline.

The agonists infused were noradrenaline bitartrate (Sigma), carbachol (Nutritional Biochemical Co.), dopamine hydrochloride (Mann), clonidine (Bohringer Ingelheim), monosodium glutamate (Sigma), and monosodium aspartate (ICN). The catecholamines were infused with 0.5 μg/μl of ascorbic acid to retard oxidation. The vehicle was distilled water or physiological saline. The order of the infusions was random.

Each rat was infused on a maximum of 4 occasions and with a minimum of 48 hrs between infusions.

Following the experiment, the brain of each rat was removed and placed in formol-saline. It was subsequently sectioned and stained with cresyl violet. The cannulas of all animals used were in close proximity to the oculomotor nucleus [1].

RESULTS

Pupillary constriction was produced by the amino acids glutamate and aspartate. The pupillary constriction generally appeared with the first μl (20 μg) and didappear within 10 to 20 min after the infusion stopped. An attempt was made to use the substance glutamic acid diethyl ester (GDEE; Sigma) to antagonize the effect of glutamate, but as shown in Table 4, no antagonism occurred.

No pupillary constriction was caused by carbachol (4.0 μg), dopamine (8.0 μg), norepinephrine (8.0 μg), or clonidine (0.3 μg). In fact, several of these agents tended to produce the reverse effect, a slight pupillary dilation. The low osmolarity of solutions containing the transmitter agonists raises the possibility that the low osmolarity was responsible for the slight dilation caused by these agents. This does not appear to be the case since infusion of saline at a similar osmolarity (0.09% NaCl) results in no substantial change from baseline. Also, carbachol produces the same effect when dissolved in 0.9% NaCl rather than distilled water (Table 4).

The dilation caused by lidocaine infusion in these animals was similar to that shown in Table 3 for the previous experiment.

GENERAL DISCUSSION

The present results systematically confirm by intracranially injections the well known pharmacological fact that transmitter antagonists can produce a nonspecific suppression of activity at some dose. With tolazoline and phentolamine nonspecific effects occur at 100 μg and 20 μg respectively using our infusion procedure. These doses are similar to those which produce hyperreactivity [4,5], feeding [16, 17, 22] and hypothermia [14,33] when injected intracranially. On the basis of dosage alone it seems quite possible that some of these reported effects could be due to a nonspecific action of these drugs. However, an additional criterion which must be used for evaluating the specificity of drug action in these cases is whether the time course of the blocking action corresponds to that known for a specific

TABLE 4

THE EFFECT OF VARIOUS TRANSMITTER AGONISTS ON PUPILLARY DILATION (mm ± S.E.M.). POSITIVE NUMBERS INDICATE DILATION. NEGATIVE NUMBERS INDICATE CONSTRICTION. TESTS WERE DONE UNDER DIM RED LIGHT. LIDOCAINE CAUSED A DILATION OF 2.0 mm OR MORE IN ALL ANIMALS WHEN TESTED IN THE LIGHT

		Changes in Dilation (± S.E.M.)	
Agonist	N	Baseline	Agonist
Glutamate (20 μg)	5	+1.8 (±0.2)	−1.2 (±0.1)
Glutamate (40 μg)	6	+2.0 (±0.0)	−1.6 (±0.1)
Glutamate (40 μg) & GDEE (60 μg)	2	+2.3 (±0.1)	−2.1 (±0.1)
Aspartate (40 μg)	5	+1.9 (±0.1)	−1.0 (±0.3)
Carbachol (4.0 μg)	4	+2.5 (±0.2)	+1.4 (±0.0)
Carbachol* (4.0 μg)	3	+0.5 (±0.0)	+1.3*(±0.1)
Norepinephrine (4.0 μg)	2	+1.7 (±1.0)	+1.3 (±0.3)
Norepinephrine (8.0 μg)	3	+2.3 (±0.2)	+0.0 (±0.0)
Clonidine (0.3 μg)	2	+1.7 (±0.3)	+1.0 (±0.0)
Dopamine (8.0 μg)	4	+2.6 (±0.3)	+0.0 (±0.0)
Saline (0.9%)	4	+2.1 (±0.2)	+0.0 (±0.6)
Saline (0.09%)	4	+2.1 (±0.3)	+0.6 (±0.4)

*Tested under normal room light.

blocking effect (compare, for example, the short lasting effect of phentolamine in Albert *et al.* [4] which appears to be nonspecific with the long lasting effects obtained by Pijnenberg *et al.* [26] which are more likely to be specific).

Pupillary dilation was produced by propranolol at doses of 20 μg when infused into the oculomotor nucleus. This result is not surprising since from other experiments propranolol is known to cause a nonspecific suppression of neural activity [10,30]. The decreased effectiveness of propranolol in anesthetized animals runs counter to the general expectation that nonspecific suppression of neural activity would be potentiated by general anesthesia. The reason for this paradoxical finding is not clear.

The blocking action of 20 μg of atropine sulfate or 10 μg of atropine methyl nitrate in the oculomotor nucleus does not appear to be specific. Evidence for a nonspecific action of atropine has previously been obtained with iontophoretic injections [9]. Behavioral experiments have found intracranial injections of atropine sulfate effective in numerous instances with doses below those at which we find nonspecific effects [13, 18, 19, 20, 28]. On the other hand, the paradoxical finding that atropine injected into the hypothalamus blocks schedule induced polydipsia [7] but not deprivation induced thirst may be due to nonspecific effects arising from the use of large doses (10 to 30 μg to each hemisphere) in the polydipsia study. Various effects found with intracranial injectin of atropine at 10 to 50 μg in several other studies may also be due to nonspecific effects [15, 29, 35]. The structural similarity of atropine and its derivatives to cocaine and procaine makes it appear likely that all will cause a nonspecific suppression of neural activity at some dosage [9].

With haloperidol, a nonspecific effect is seen at 20 μg. In agreement with the present findings, haloperidol has been found to block conduction along sciatic and phrenic nerve axons when bathed in solutions with concentrations as low as 10^{-7}M/1 [31]. In contrast, the 5 μg/ul infused in the present experiment represents a concentration in the order of 10^{-1}M/1. The intracranial dose of haloperidol at which substantial nonspecific suppression of neural activity appears (10 μg) is higher than that commonly used for intracranial injections [6, 8, 10, 11, 18, 24, 25].

GENERAL COMMENTS

The present method appears to produce a valid indication of the dosage at which nonspecific suppression of neural activity might begin to appear with slow intracranial infusions. In this respect they complement the classical pharmacological procedure of determining whether transmitter agonists and antagonists produce opposing effects. As was evident in the present experiment (Table 4), agonists may produce confusing results of their own for uncertain reasons. It seems certain that the time course of the nonspecific suppression of activity will differ from the duration of transmitter specific blocking effects. Accordingly, it seems particularly important to gather data on the time course of behavioral effects induced by transmitter antagonists as an additional means of evaluating the specificity of action produced by a drug. In this regard, it should be noted that injection of a local anesthetic is not an adequate control for nonspecific effects of transmitter antagonists [15,24] because the duration of the nonspecific suppression of activity produced by transmitter antagonists can be entirely different from the duration of the block produced by the local anesthetic.

Nonspecific effects of transmitter antagonists appear to be affected by the concentration of a drug and the injection procedure as well as the dose. This was verified in pilot studies in the course of the present experiment. These pilot experiments suggested that the slow infusion procedure used in the present experiment facilitates uptake of the pharmacological agent in the vicinity of the infusion needle tip and may in that way increase the incidence of nonspecific

effects in comparison to pulse injections of more concentrated solutions (0.5 to 1.0 μl over 2 to 5 sec). Accordingly, the nonspecific effects of a drug must be evaluated under the conditions in which it is to be used. The doses at which nonspecific effects appeared in the present experiment can only be taken as a very rough guideline and precise estimations will have to be made using the mode of injection intended for any particular behavioral experiment.

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