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BRIEF COMMUNICATION

The Rabbit Ear-Withdrawal Test: A New Analgesiometric Procedure

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McCALLISTER, L. W., J. M. LIPTON, A. H. GIESECKE, JR. AND W. G. CLARK. *The rabbit ear-withdrawal test: A new analgesiometric procedure.* PHARMACOL BIOCHEM BEHAV **25**(2) 481–482, 1986.—The latency to movement of an ear exposed to radiant heat was prolonged after intravenous administration of morphine to rabbits. The quantification of this response in a relatively inactive species that is especially suited for long term and repeated tests suggests that the rabbit ear-withdrawal test will be useful for screening analgesic/anesthetic compounds.

Analgesiometry Pain model Rabbit Ear-withdrawal Morphine

PERHAPS in large part because of their relatively small size and low cost, rodents almost exclusively have been used in screening procedures for analgesic/anesthetic agents. The rat tail-flick test [1], the mouse hot-plate test [4] and various chemically-induced writhing assays are common examples of such procedures [3]. The use of these tests has expanded our basic knowledge of the algesic/analgesic properties of various substances, and they have provided firm foundations for clinical testing. However, in many screens the stress of restraint on the normally active rodents will undoubtedly influence the results. Also, such screens may not be appropriate for tests of intravenously administered agents, especially if repeated injections or long term infusions are required. Since rabbits are generally much less active than rodents, tolerate restraint for prolonged periods and have large lateral ear veins that can easily be cannulated or injected into directly, this species was tested for the response to application of radiant heat to the ear. The aim was to learn whether a reliable ear-withdrawal response could be established as a base line against which the effects of analgesics could be assessed. The prototype opiate analgesic morphine was given intravenously to assess the ear-withdrawal reaction.

METHOD

Male New Zealand white rabbits (3–4 kg, Hickory Hill) were habituated to the laboratory environment and to restraint in standard stainless-steel rabbit holders for 1 week prior to testing. The anterior surface of the base of both ears was shaved and blackened with permanent marker ink to reduce insulation, to localize heating primarily to the surface and to reduce variability in reflection due to slight differences in coloration among animals.

The apparatus (Fig. 1) consisted of a heat source, a projector lamp (150 W, 120 V), situated directly behind a brass shield with a $^{7}/_{16}$ inch diameter hole through which the radiant heat projected. The heat source was held in position relative to the ear by a pantograph arm. A 1.5 inch long plastic rod was attached to the shield to serve as a guide for consistent positioning of the lamp relative to the ear. The heat source and a signal marker on a polygraph (Grass Instruments Model 7B) were controlled by the same hand-held push-button switch. When an ear movement occurred, generally either a rapid, short flick or a complete movement out of the light path, the push-button was released, thus turning off the heat source and simultaneously indicating the response latency on the polygraph record. Each duration of exposure to heat was determined directly from the polygraph record.

Three rabbits were first tested after IV administration of morphine, dissolved in 0.10 ml saline. One week later the test was repeated after a control injection of saline. The order of treatment was reversed for three other animals. Before each experiment the restrained rabbits rested in the experimental room for 30 min; then the response to heat was

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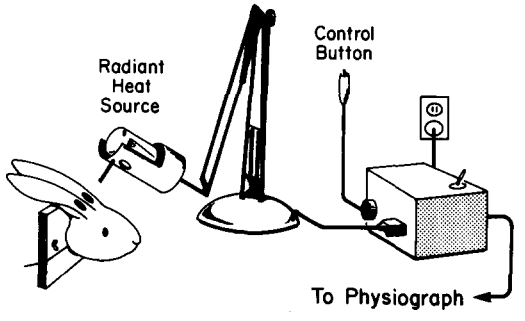

FIG. 1. Schematic diagram of the rabbit ear-withdrawal analgesiometric apparatus.

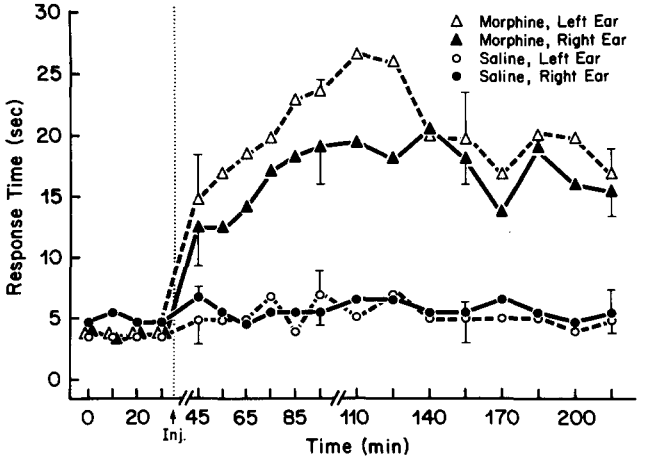

FIG. 2. Effect of intravenous administration of morphine sulfate (2.5 mg/kg) or saline on latency to ear-withdrawal in left and right ears of six rabbits. Injections were given, as indicated by the arrow, after a period of about 30 min to establish the base line.

tested at 10-min intervals. Both ears were tested at each interval, in random order. After the fourth base-line test, saline or 2.5 mg/kg morphine sulfate was injected into a lateral ear vein of the left or right ear, again assigned randomly. Latency to ear movement was recorded at 10-min intervals for 1 hr post-injection and at 15-min intervals for the next 2 hr. To prevent tissue injury, a time limitation for exposure to heat was set at five times the average pre-injection response time. In a separate study saline and 1.25 mg/kg morphine sulfate were also tested.

RESULTS AND DISCUSSION

The latency to ear movement during pre-injection tests was very consistent among animals, averaging 4–5 sec for each ear (Fig. 2). Whereas saline injection had no effect on latency to ear-withdrawal, morphine sulfate (2.5 mg/kg) caused a 4 to 5-fold maximum increase approximately 1–1.5 hr post-injection. There was no significant difference between the responses of the two ears regardless of which had received the injection ($p \geq 0.20$, Wilcoxon test). When a smaller dose of morphine (1.25 mg/kg) was tested similarly in six animals, it increased the latency to response approximately three-fold (mean=3.08-fold increase).

These initial findings suggest that the rabbit ear-withdrawal response to radiant heat may be a useful screen for analgesic agents. Saline injection into the ear vein did not markedly alter the response latency, which remained quite consistent for 3 hr. Under the conditions used there was a considerable breadth of latency from which to choose a specific time for a standardized test, and a lower dose of morphine had less effect than the standard 2.5 mg/kg dose. Although the time to peak analgesia may seem somewhat long, it is consistent with the latency to maximal change in another physiologic parameter, i.e., body temperature, when a slightly larger dose of morphine was given to rabbits by the same route [2]. These observations indicate that the rabbit ear-withdrawal test should be a simple, convenient and reliable analgesiometric procedure that may offer an alternative to rodent models. Additional experiments with other opioid compounds, with non-steroidal anti-inflammatory drugs and with anesthetic agents will be required to fully evaluate the usefulness of this technique for assessing analgesic activity and for discriminating among different doses of analgesic compounds.

It should be mentioned that radiant heat was chosen in pilot research as the noxious stimulus as opposed to electrical stimulation. The latter involves a potential tactile interference from electrode attachment, plus uncontrollable variability of stimulation and of response which occurs as a result of incorporation of body tissue into an electrical circuit. The radiant heat stimulation used in the ear-withdrawal test also has the humane advantage of allowing the animal to withdraw from the source of irritation.

ACKNOWLEDGEMENT

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