Pharmacology Biochemistry & Behavior, Vol. 4, pp. 481–483.

BRIEF COMMUNICATION

Stimulus Specific Effect of Scotophobin on Mouse Plasma Corticoids

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(Received 7 July 1975)

MALIN, D. H., G. J. RADCLIFFE, JR. AND D. M. OSTERMAN. *Stimulus specific effect of scotophobin on mouse plasma corticoids.* PHARMAC. BIOCHEM. BEHAV. 4(4) 481–483, 1976. – Scotophobin is a peptide previously extracted from brains of rodents shocked in the dark compartment of a white/dark apparatus and identified as the behaviorally active dark avoidance-inducing factor. This study attempted to ascertain whether a stable synthetic analog of scotophobin induced no adrenocortical stress reaction, a generalized, unconditioned stress reaction, or a stress reaction selectively triggered by the dark compartment of the apparatus. Twenty eight mice were injected IP with the synthetic substance and 28 with placebo. A day later half of each group was placed in the dark compartment and the other half in the light compartment. Fifteen min afterward, the animals were bled, and serum samples were obtained. Plasma corticoid levels were determined by radioassay. Only the animals injected with scotophobin analog and exposed to the dark box had elevated levels. The interaction effect between drug treatment and light or dark environment was highly significant.

Scotophobin Corticosteroids Stress Memory Stimulus specificity

SCOTOPHOBIN is a brain peptide chemically detectable in rodents trained to avoid the dark compartment of a light/dark apparatus by being shocked repeatedly in that compartment [9,11]. Natural scotophobin or a synthetic version of it, injected into naive mice, causes them to spend significantly less time in the dark compartment than control mice when given a free choice [4,11]. This effect generally begins within a day after injection and may last almost a week.

Ungar interprets this chemically induced avoidance behavior to mean that scotophobin, like the actual shock training, induces a conditioned fear specific to the dark box. This, in turn, could suggest that the observed biosynthesis of scotophobin following such training may help mediate memory formation for this specific learned fear.

However, Goldstein [2] has suggested that the behavioral dark avoidance shown by scotophobin recipients may somehow result from a general, unconditioned, non-stimulus specific stress, anxiety or arousal produced by scotophobin.

The purpose of the experiment reported here was to determine whether scotophobin produces no stress, a general stress, or a stimulus specific stress reaction as measured by plasma corticoid levels, an index of the adrenocortical stress reaction.

METHOD

The behavioral apparatus has been described in detail by Ungar [10] and Malin [3]. Basically, it consisted of 3 Plexiglas compartments, connected by short corridors with guillotine doors. The central white compartment was flanked by a smaller dark compartment on one side and a smaller white compartment on the other.

A stable synthetic analog of scotophobin was synthesized by D. M. Desiderio by the classical method. This substance appears to be chromatographically similar to the pentadecapeptide synthesized by Weinstein and shown by de Wied to prevent extinction of passive avoidance of a dark compartment [1]. At a dose of 4 μg IP, it causes a degree of dark avoidance in mice similar to that produced by the optimal dose (0.67 μg) of scotophobin synthesized by Parr [8] by the solid-phase method, not available at the time of this experiment. Ungar [12] discusses the several scotophobin preparations.

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TABLE 1

CONCENTRATION OF MOUSE PLASMA CORTICOIDS AS AFFECTED BY SCOTOPHOBIN AND 3 MINUTE EXPOSURE TO LIGHT AND DARK COMPARTMENTS

Experiment*	Injection	Plasma Corticoids ng/ml ± S.E. White Box	Black Box
A. Main Experiment	Distilled water	133±19	92±14
	Scotophobin (Desiderio) 4 μg	117±11	209±25
B. Pilot Experiment	Distilled water	100	90
	Scotophobin (Parr) 0.5 μg	97	148

*14 mice per group. In Experiment A, estimations were done in individual samples; in Experiment B, the samples of each group were pooled.

Animals were 56 mature male mice, from 26 to 28.5 g, screened for normal dark preference (at least 90 sec spent in dark during a 3 min free choice period). The mice were habituated to the apparatus by being placed in it for 3 min a day for 10 days. The animals were randomly divided into 4 groups of 14 mice each. In accordance with the method of Ungar [10], animals in Groups 1 and 2 each received an IP injection of 4 μg scotophobin analog in 0.25 ml distilled water. Animals in Groups 3 and 4 received 0.25 ml distilled H_2O. Twenty-four hr after injection, mice in Groups 1 and 3 were locked for 3 min in the dark box. Mice in Groups 2 and 4 were similarly locked for 3 min in the white compartment of similar size on the other end of the apparatus. There were thus 4 treatment groups: Scotophobin/dark box, scotophobin/light box, control/dark box, and control/light box.

Fifteen min after removal from the apparatus each mouse was bled into a heparinised beaker. Plasma was obtained by centrifuging for 1 min in a clinical centrifuge. Approximately 0.5 ml plasma was obtained per mouse. The individual samples were coded and assayed on a blind basis for corticoid levels by the standard radioassay method of Murphy [6,7]. The principle of this method is that known amounts of corticoid binding protein and radioactive corticoid standards are incubated with an aliquot of plasma sample. Competitive inhibition of the binding of the radioactive standards is measured, allowing determination of plasma corticoid levels. The corticoid estimation was done on an automated basis in the Pediatric Endocrinology Laboratory of Baylor College of Medicine, whose personnel received the plasma samples under a code number and were not informed of the nature of the experiments. Estimations were done in duplicate samples.

An earlier pilot experiment used identical methods except in 2 respects: Parr's scotophobin preparation was used at a dose of 0.5 μg, and all samples within each treatment group were pooled, precluding statistical tests of significance.

RESULTS

Table 1A summarizes the results of the main experiment. In cases where the animal was exposed to the light box before bleeding, scotophobin produced no increase in corticoid levels. (In fact, the scotophobin mice averaged 12% lower). In mice exposed for 3 min to the dark box, however, scotophobin produced a 125% average increase in corticoid level.

A 2 x 2 analysis of variance disclosed a highly significant interaction effect between the variables of test environment and scotophobin administration ($p<0.001$), indicating that the stress producing effect of scotophobin is strongly dependent on the environment to which its recipient is exposed. The scotophobin/dark group is significantly different from both the scotophobin/light group ($p<0.005$) and the control/dark group ($p<0.01$) according to Duncan's Range test for multiple post hoc comparisons.

Table 1B shows the corticoid levels of the 4 pooled samples obtained in the pilot experiment using Parr's scotophobin. It is interesting that the scotophobin/dark group again had the only elevated level, although no statistical conclusions can be drawn due to the use of pooled plasma samples.

DISCUSSION

Apparently, scotophobin can produce an adrenocortical stress response, if the recipient is exposed to the same dark compartment where animals are punished to acquire dark avoidance (and to induce scotophobin synthesis in their brains). This seems to fit in with the results of an earlier experiment of somewhat similar design using defecation rate as an index of stress or anxiety. Scotophobin significantly increased defecation rate in the dark compartment but not in the white compartment or a transparent compartment. Again there was a highly significant interaction effect between drug treatment and test environment [3]. The most direct explanation of all these findings is that scotophobin, a substance synthesized in rats shocked in a dark box, causes a stress or fear reaction to a dark box environment. Another explanation would assume that scotophobin simply reduces visual acuity under dim illumination and that animals that do not see well in the dark have a stress reaction to dark environments. Malin and Radcliffe [5] found, however, that the illumination threshold for visually guided performance was no higher for scotophobin treated animals than for controls.

All of these findings, together with others reported by Malin [3], show that it has been very difficult thus far to operationally differentiate the effects of scotophobin from a mild conditioned anxiety or aversion to a dark compartment.

ACKNOWLEDGEMENTS

Thanks to B. S. Keenen for hormone determinations, to G. Ungar and A. L. Ungar for helpful suggestions and Faye Johnson for skilled assistance.

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