Neuroscience Letters, 163 (1993) 177-181

NSL 09981

Photic induction of Fos in the hamster suprachiasmatic nucleus is inhibited by baclofen but not by diazepam or bicucullin

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(Received 26 June 1993; Revised version received 12 August 1993; Accepted 17 August 1993)

Key words: Bicuculline; Baclofen; Circadian; Diazepam; Fos; GABA; SCN

The present study makes use of the photic induction of Fos in the suprachiasmatic nucleus (SCN) to explore the pharmacology of retinal input to this circadian pacemaker. Our results demonstrate that the $GABA_A$ antagonist bicuculline and the benzodiazepine agonist diazepam, both of which prevent light-induced phase shifts, do not inhibit photic induction of Fos expression in the hamster SCN. In contrast, the $GABA_B$ agonist, baclofen, prevents both light-induced phase shifts and inhibits photic induction of Fos expression in the SCN. One explanation of this difference may be that baclofen acts to prevent photic information from reaching the SCN while bicuculline and diazepam act within the SCN at a point 'downstream' from Fos induction.

In mammals, the suprachiasmatic nucleus (SCN) is the dominant pacemaker of the circadian system. To function adaptively, the SCN must be synchronized (or entrained) to the environment. The daily light-dark cycle, acting through light-induced advances and delays of the endogenous oscillation, is the primary environmental entraining agent. The effects of light on the SCN are mediated by photoreceptors which are located in the retina and project to the hypothalamus, at least in part, via a monosynaptic fiber tract known as the retinohypothalamic tract (RHT) [11].

Photic regulation of c-*fos* mRNA and Fos-like immunoreactivity (Fos-LI) in the SCN of rodents has been extensively demonstrated [2, 4, 5, 7, 16, 17]. Previous studies have shown that photic regulation of Fos in SCN neurons is correlated with light-induced phase shifts of the circadian system. For example, induction of c-*fos* mRNA by light in the hamster SCN shows the same phase dependence and intensity threshold as does phase shifting [7]. Collectively, this work suggests that Fos induction is a useful cellular marker of photic input to the SCN.

Previous studies have shown that pharmacological agents which alter γ-aminobutyric acid (GABA) receptor function can prevent light-induced phase shifts of the circadian rhythm of locomotor activity in hamsters. The $GABA_A$ antagonist bicuculline prevents light-induced phase delays, but not light-induced phase advances [13]. Conversely the benzodiazepine diazepam, which can potentiate GABA activity, blocks light-induced phase advances but not delays [14]. In addition, the $GABA_B$ receptor agonist baclofen prevents both light-induced phase advances and delays [15]. In the present study, we investigated the effect of these agents on light induction of Fos-LI in the hamster SCN.

Adult male hamsters (*Mesocricetus auratus*, LVG-outbred, Charles Rivers, Wilmington, MA) were housed individually and their wheel-running activity recorded. Animals were exposed to a light-dark cycle of 12 h light:12 h dark for 2 weeks at which time they were placed in constant darkness for at least 10 days to assess their free-running locomotor activity period.

The light stimulus used in these experiments was a 15 min pulse of monochromatic light (515 nm; half-peak bandwidth 10 nm) at an irradiance of 1×10^{-1} $\mu W/cm^2$. Bicuculline (4.0 mg/kg), in isotonic saline, was administered by i.p. injection (volume was 0.2 ml) 5 min prior to the light pulse. Diazepam (12.5 mg/kg), in saline and 50% dimethylsulfoxide, and baclofen (15.0 mg/kg), in saline and lactic acid were administered by i.p. injection 30 min prior to the light. Pilot experiments confirmed that bicuculline (3.6 mg/kg) blocks light-induced phase delays in the circadian rhythm of wheel-running activity, diazepam (12.5 mg/kg) blocks phase advances, and that ba-

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clofen (15 mg/kg) blocks both advances and delays. The light treatment used produces phase delays in the circadian rhythm of wheel-running activity when delivered at circadian time (CT) 13.5 or phase advances when delivered at CT 18 (the onset of locomotor activity in constant darkness is defined here as CT 12). Following the treatments, the hamsters were returned to constant darkness for 60 min until perfusion. All handling and treatment of animals before perfusion was carried out in complete darkness with the aid of an infrared viewer (FJW Industries, Elgin, IL). Bicuculline and diazepam were purchased from RBI (Natick, MA) while baclofen was purchased from Sigma (St. Louis, MO).

Immunohistochemical methods used have been previously published [4]. The antisera used in this study was an anti-Fos rabbit polyclonal antiserum (Oncogene Science, Uniondale, New York; dilution 1:250) while binding sites were visualized with an biotin-avidin-peroxidase procedure (Elite ABC kit, Vector Labs, Burlingame, CA). Differences between treatment groups were evaluated using a Kruskal-Wallis one-way analysis of variance followed by a Mann-Whitney *U*-test where appropriate. Values were considered significantly different if $P < 0.05$.

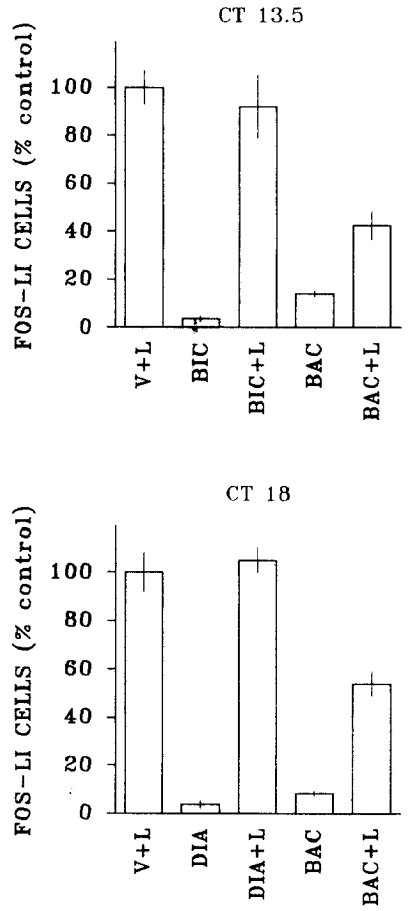

Fig. 1. Baclofen, but not bicuculline or diazepam, inhibited light-induction of Fos-LI. Results from experimental and control groups are expressed as a mean percentage relative to the number Fos-positive cells in the SCN of vehicle and light controls. In the top panel light pulses were delivered at CT 13.5 while in the bottom panel light pulses were delivered at CT 18. At both phases, the experimental group treated with baclofen and light (BAC+L) was significantly different ($P < 0.05$) from the vehicle and light control group. In contrast, the experimental groups treated with diazepam and light (DIA + L) or bicuculline and light (BIC + L) were not significantly different from the controls. $n = 4$–8 for all points, vertical bar represents S.E.M.

The administration of the $GABA_A$ receptor antagonist bicuculline or the benzodiazepine agonist diazepam did not inhibit light induction of Fos-LI in the SCN (Figs. 1 and 2). Hamsters which were injected with vehicle and exposed to light at either CT 13.5 ($n = 5$) or CT 18 ($n = 4$) also showed robust Fos-LI in the SCN. The pattern of staining was similar to that which has been previously described [24], with most staining occurring in the ventrolateral region of the SCN. Control injections of bicuculline, diazepam, or vehicle administered without a subsequent light pulse did not lead to a significant effect on Fos-LI at either of the phases ($n = 4$–5 at both phases). The administration of bicuculline or diazepam did not alter the number or distribution of light-induced Fos-LI in the SCN.

In contrast, the administration of the $GABA_B$ receptor agonist baclofen inhibited photic induction of Fos-LI in the SCN (Figs. 1 and 3). Hamsters which were injected with vehicle and exposed to light at either CT 13.5 ($n = 4$) or CT 18 ($n = 5$) showed robust staining in the SCN. Administration of baclofen alone, but not vehicle controls, caused a small but consistent increase in Fos-LI in the SCN ($n = 4$ at each phase). In addition, baclofen inhibited, but did not eliminate, light-induced Fos-LI in the SCN at both CT 13.5 and CT 18 ($n = 4$ per group). Interestingly, there were consistent regional differences in the effects of baclofen: this antagonist did not prevent photic induction of Fos-LI in a population of cells in the caudal mediolateral region of the SCN (8/8 animals).

The present study makes use of Fos expression in the SCN as a tool to explore the pharmacology of retinal input to the SCN. Our results demonstrate that the administration of baclofen, but not bicuculline or diazepam, inhibits photic induction of Fos-LI in the hamster SCN. Most of the light stimulated Fos-LI in the SCN occurs in the ventrolateral region of this nucleus – much of this staining was inhibited by baclofen. However, there was a population of cells in the caudal mediolateral SCN where Fos-LI was light-induced but was baclofen-insensitive. Interestingly, previous workers have also reported pharmacological differences between the photic induction of Fos-LI in different regions of the SCN [1]. These findings suggest that different receptor types mediate photic induction of Fos-LI in different regions of the

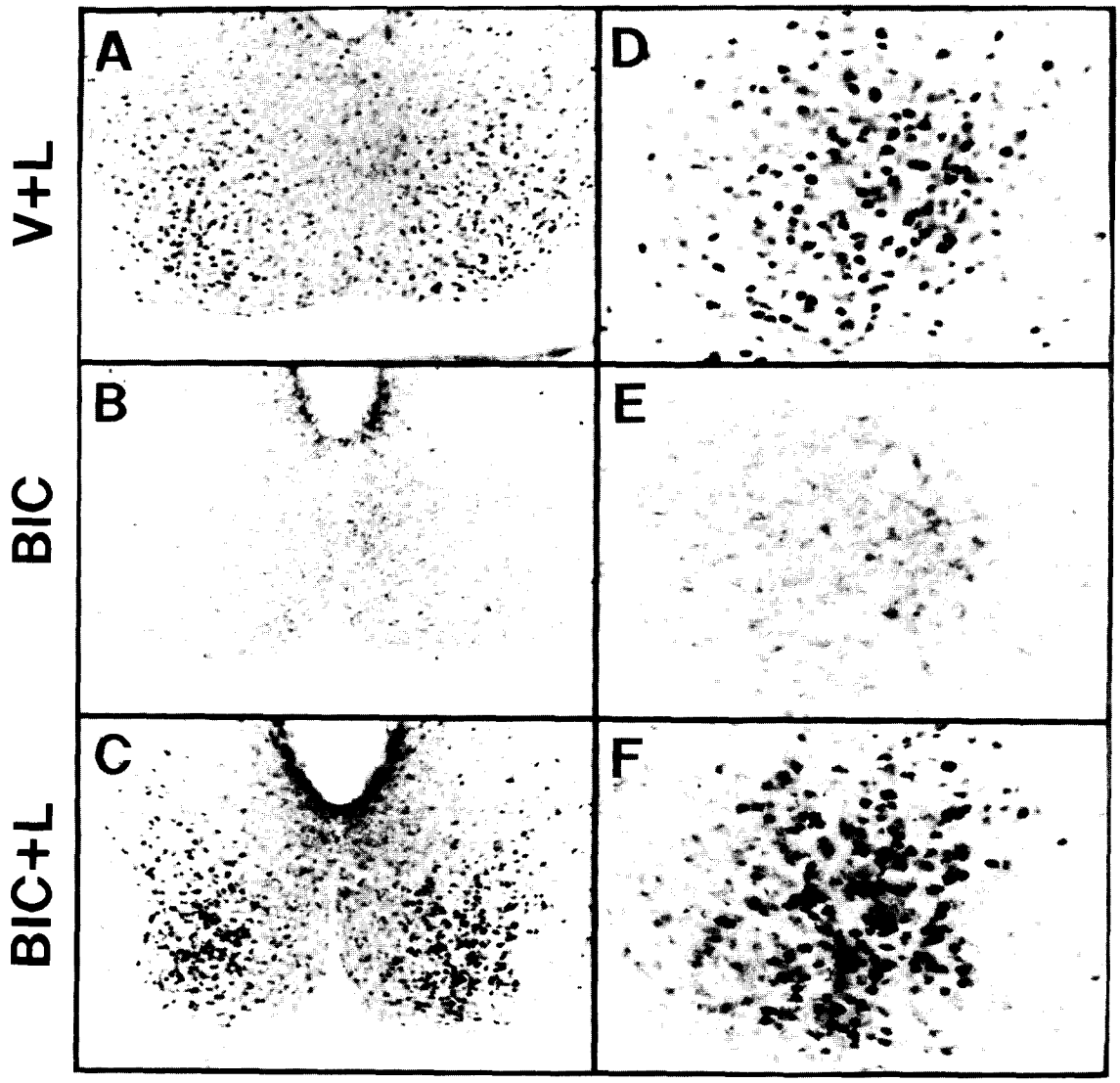

Fig. 2. Bicuculline does not inhibit photic induction of Fos-LI in the hamster SCN. Photomicrographs are of coronal sections through the central SCN region which have been stained for Fos-LI. Hamsters in constant darkness were treated with either vehicle and light (A,D), bicuculline alone (B,E), or bicuculline and light (C,F). Animals were exposed to light at CT 13.5. Magnification 200 × (A–C) and 400 × (D–F).

SCN. In addition, since the administration of baclofen prevents light-induced phase shifts, the Fos induction in the mediolateral SCN may not play a role in mediating light-induced phase shifts.

Earlier behavioral studies suggest that GABA receptors are involved in the photic regulation of the circadian system and raise questions about the neuroanatomical site(s) of their action. In order to address these questions, we assumed that light causes a linear cascade of events resulting in a phase shift of the circadian oscillator in the SCN and a concomitant increase in Fos expression. GABAergic agents might affect synaptic transmission (or other biochemical events) at any point(s) along this pathway. If these agents were acting at a point on the light-input pathway before the signal reached the SCN, one would expect that they would inhibit photic induction of Fos. In our experiments, this was the case with baclofen but not with bicuculline or diazepam. Our findings suggest that baclofen is acting to prevent photic information from reaching the SCN while bicuculline and diazepam act to prevent light-induced phase shifts at a point 'downstream' from Fos induction.

Several pieces of evidence support the suggestion that the GABAergic drugs bicuculline and diazepam are acting directly within the SCN to prevent light-induced phase shifts. Anatomical studies indicate that GABA and the GABA synthetic enzyme, glutamic acid decarboxylase, are found throughout the SCN [3, 12, 19]. There is also evidence of diazepam binding sites in the SCN [10]. In addition, electrophysiological studies have shown that GABAergic agents can influence the electrical activity of SCN neurons [e.g. 6, 8, 9, 18]. For exam-

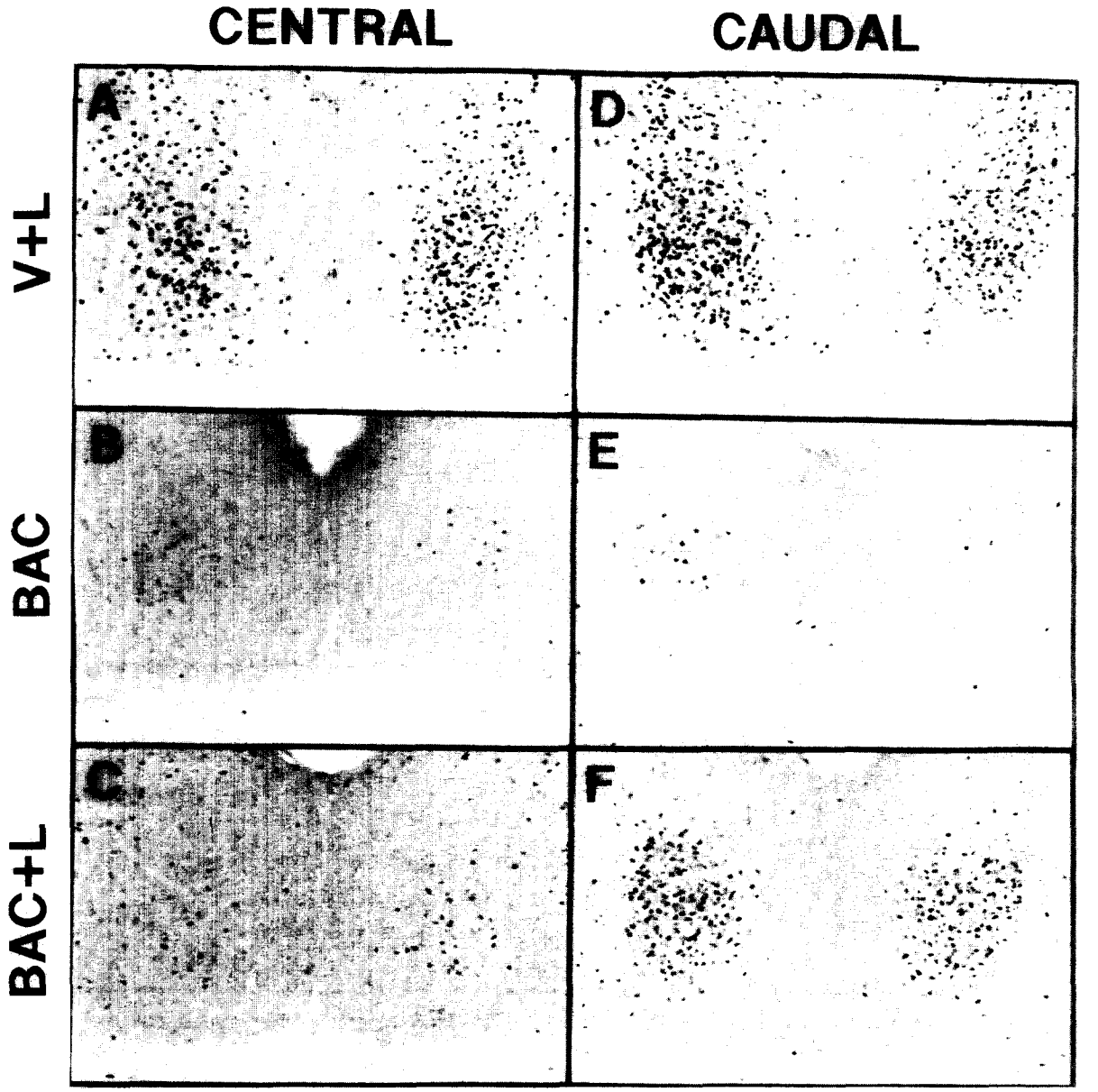

Fig. 3. Baclofen inhibits photic induction of Fos-LI in the hamster SCN. However, this effect was regionally specific as baclofen did not inhibit light regulated Fos-LI in a population of cells in the caudal mediolateral SCN. Photomicrographs are of coronal sections through the central and caudal SCN region which have been stained for Fos-LI. Hamsters in constant darkness were treated with either vehicle and light (A,D), baclofen alone (B,E), or baclofen and light (C,F). Animals were exposed to light at CT 18. Magnification 200 ×.

ple, a study by Kim and Dudek using a hypothalamic brain slice preparation has shown that bicuculline blocked inhibitory postsynaptic potentials in SCN neurons [6]. While the neuro-anatomical sites of action of bicuculline and diazepam remain uncertain, our results make it unlikely that their blockade of phase shifting occurs in the retina or at the RHT/SCN synapse. We suggest that these agents act within the SCN at a point 'downstream' from or not directly related to Fos induction.

In this study, we have used the photic induction of Fos-LI in the SCN as a tool to gain information about possible sites of action of three GABAergic agents which are known to prevent light-induced phase shifts. Our results reveal complexity in the pharmacology of photic input to the SCN. First, among the SCN cells in which Fos is light regulated, there exist both baclofen-sensitive and baclofen-insensitive populations. Second, our results demonstrate that $GABA_B$ but not $GABA_A$ receptors can influence photic induction of Fos. This later results suggests that although baclofen, diazepam, and bicucullin can all prevent light-induced phase shifts, they do so through at least two different mechanisms. An alternative explanation is that Fos induction is not part of the

signal transduction cascade which results in phase shifts and that the observed correlations are epiphenomenal.

We would like to thank Dr. N. Wayne for comments on an early draft of this paper. This work was supported by NIH Grant HD13162 to M.M. C.M.K. was supported by the NSF Center for Biological Timing while C.S.C. was supported by a FESN Fellowship in 'Circadian Rhythms'.

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