# EXPERIMENTAL BIOLOGY

# Investigation of Transcription in Nerve Cells and Hepatocytes of WAG and F344 Rats of Different Ages

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 122, No. 10, pp. 427-431, October, 1996
Original article submitted July 2, 1995

The effects of morphine and the protease inhibitor antipain on the RNA synthesis in nerve cells and hepatocytes are studied in rats of two inbred strains (WAG and F344) differing in narcologic resistance and age. The effects of morphine and antipain on the intensity of RNA synthesis are assessed by incorporation of $^3$H-uridine into the neurons of sensorimotor cortex, hypothalamic ventromedial nucleus, blue spot, superior, and superficial cervical ganglion and in hepatocytes. These agents produce different effects on transcription in WAG and F344 rats.

**Key Words:** *narcologic resistance; morphine; postnatal ontogenesis; transcription*

Elucidation of molecular mechanisms underlying the effects of morphine is important for the development of new therapies of the dependence on morphine and its derivatives. Altered transcription represents one of these mechanisms. The template activity and chemical composition of chromatin change with age [3,4]. The intensity of morphogenetic processes is the highest during embryogenesis and early postnatal ontogenesis, it declines in adults, while in aged people the rate of RNA synthesis is markedly decreased and genetic repression in different organs and tissues, for example, neurons and hepatocytes, is increased [3,8]. Age-related variations of the response to morphine have been documented [8]. The response to morphine was studied in rats aged 3 and 14 days and 1, 6, and 12 months, i.e., at ages when transcriptional changes in the sympathetic neurons were reported to be maximal [9].

The density of opiate receptors is high in brain structures with a high content of endogenous opioid peptides. These structures are involved in the development of addiction to alcohol and narcotics [5]. They have high contents of serotonin-, dopamine-, and noradrenergic neurons. A strong morphofunctional relationship between monoamine and opiate brain systems has been described [5]. Disturbances in these systems may promote transformation of alcohol and drug addiction into a morbid state as well as development of physical dependence.

There is evidence that predisposition to drug and alcohol abuse, tolerance, and dependence are genetically controlled both in humans and animals [7]. It was hypothesized that genetic differences in the metabolism of monoamines and endogenous opioids determine individual variations of the response to narcotics.

Our goal was to investigate the age-related variations in the rate of RNA synthesis in neurons and hepatocytes of F344 and WAG rats differing in the resistance to narcotics.

## MATERIALS AND METHODS

Hepatocytes and neurons of the superior cervical ganglion (SCG), sensorimotor cortex (SMC), ventromedial nucleus (VMN) of the hypothalamus, and blue spot of F344 and WAG rats ($n$=100) aged 3 and 14 days and 1, 6, and 12 months were studied. Persisting sections were incubated by conventional

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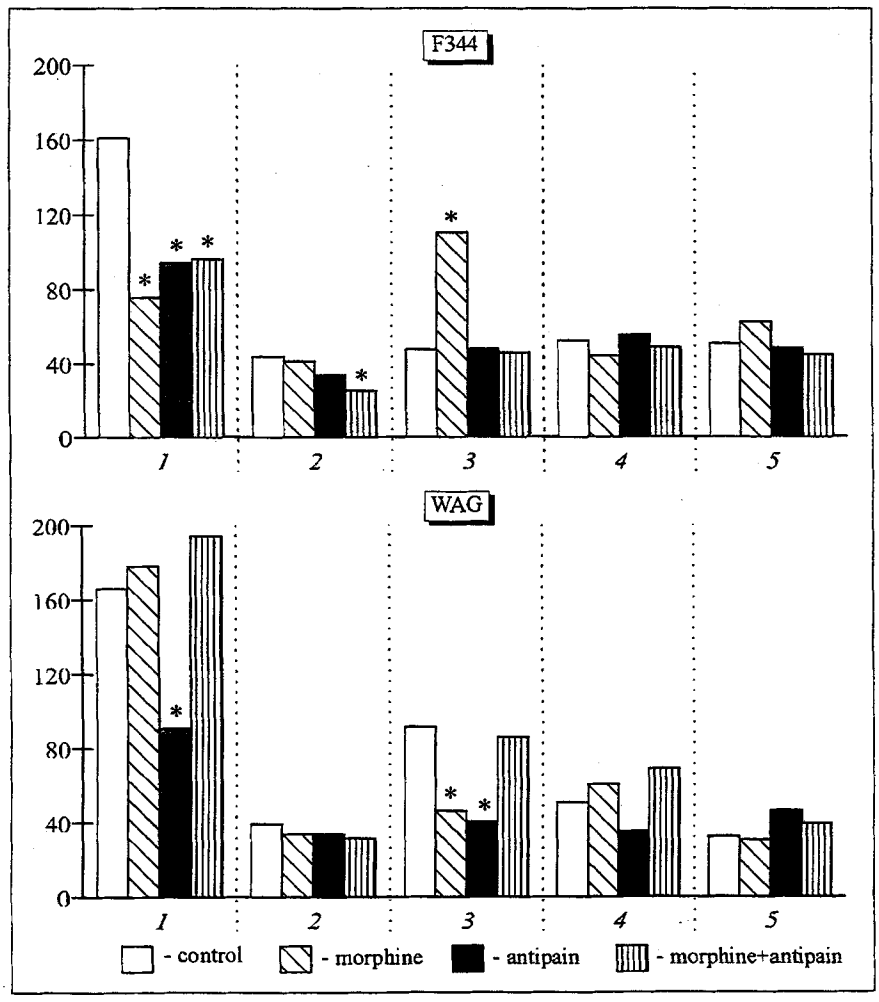


**Fig. 1.** Incorporation of $^3$H-uridine in total RNA of neurons and hepatocytes of 3-day-old F344 and WAG rats injected with morphine and antipain. Here and on Figs. 2-5: abscissa: relative incorporation of the label in RNA molecules; ordinate: intensity of scintillation, cpm/mg wet weight. *$p<0.05$ compared with control.

method [2] with morphine, the protease inhibitor antipain (final concentrations in the incubation media 8 and 0.08 mM, respectively), and $^3$H-uridine. Control sections were incubated in medium without these agents. Results were expressed as specific radioactivity of the sections (cpm/mg wet weight). Determination of absolute and relative incorporation of the radiolabel in RNA was described previously [2]. Data were processed using Student's *t* test.

## RESULTS

The results obtained confirm that transcriptional activity changes with age [3,4,9]. It was low in all studied structures of 3-day-old rats of both strains, except the SCG (Fig. 1). This may be due to differentiation of some elements of the studied structures in early ontogenesis. In 14-day-old animals, transcriptional activity increased considerably in all structures, except blue spot and hepatocytes of WAG rats, reaching the maximum in the SCG of F344 rats and VMN of WAG rats (Fig. 2). Transcriptional activity in the SCG of F344 rats aged 1 and 12 months was higher than in other structures. The same was true for 1-month-old WAG rats; in 6-month-old WAG rats, incorporation of $^3$H-uridine in the SMC neurons was the highest (Figs. 3-5). In control rats, transcriptional activities were significantly different in the SCG of 3-day-old rats; in the SCG, blue spot, and hepatocytes of 14-day animals; and in the SCG and VMN of 1- and 12-month-old rats. This may reflect genetic differences of animals with different sensitivities to narcotics.

Our findings indicate that neuronal response to morphine changes with age in all studied structures of F344 and WAG rats, except the SMC of F344 rats, where no significant changes in $^3$H-uridine incorporation were observed. Morphine potentiated or suppressed transcription, the effect being sometimes opposite in the same structures of rats of different age (SCG in both strains and SMC in WAG rats). It is noteworthy that the reaction of nerve cells to morphine was the same in 14-day-old and 1-month-old rats of both strains, with the exception of the blue spot neurons of F344 rats. In 1-month-old rats, there were no strain-specific differences in the morphine reaction of the studied structures, except that of the SMC neurons.

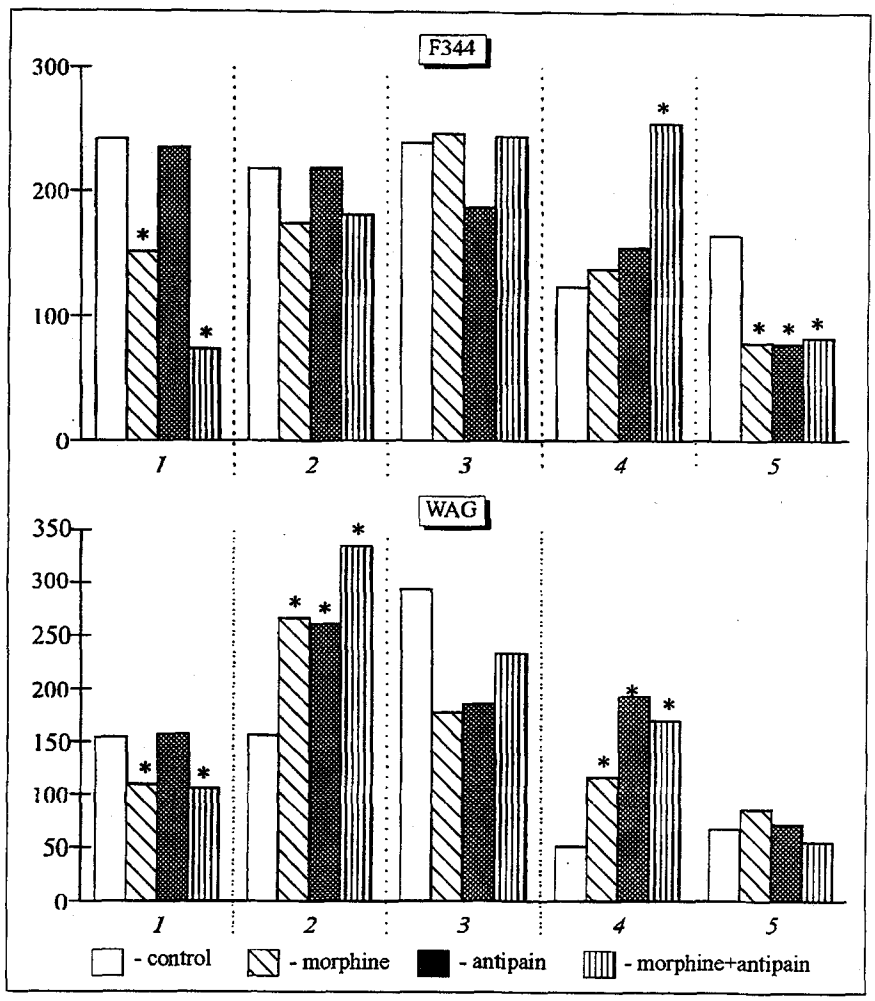


Fig. 2. Incorporation of $^3$H-uridine in total RNA of nerve cells and hepatocytes of 14-day-old WAG and F344 rats injected with morphine and antipain.

The effect of antipain on SMC, VMN, and blue spot neurons of 3- and 14-day-old WAG rats was the same as that of morphine. In the same structures of age-matched F344 rats antipain did not change significantly the label incorporation (Figs. 1 and 2). A similar response of SCG was observed in both strains. At later stages of ontogenesis (1, 6, and 12 months), the effects of both drugs on the synthesis of RNA were the same in a number of structures (Figs. 3-5). Antipain caused no changes in SCG and VMN of adult F344 rats.

The effect of morphine on hepatocytes (Figs. 1-5) was the same as that of antipain alone and in combination with morphine observed in all age groups. The effects of morphine and antipain on transcription in hepatocytes of young and adult WAG rats did not differ from the control, while in old rats both agents stimulated transcription. At the same time, both preparations suppressed the label incorporation in 14-day-old F344 rats and stimulated it in 6-month-old rats. So far, it is difficult to make any conclusion from this finding.

The effect of the morphine-antipain combination on transcription implies the existence of a protease mechanism of the RNA synthesis modulation in response to morphine [1]. Administration of antipain in combination with morphine abolished the effect of morphine on the VMN neurons in 3-day-old rats. This was observed in the SCG and VMN of 6-month-old F344 rats and in the blue spot of 1- and 12-month-old F344 rats as well as in the SCG and blue spot of 1- and 6-month-old WAG rats and in the VMN of 12-month-old WAG rats. In the majority of animals, morphine-induced changes disappeared. It should be noted that after combined administration of morphine and antipain, the effect of morphine was abolished in the same structures (SCG, blue spot, and VMN) of F344 and WAG rats of different age. The number of noradrenergic neurons in these structures is particularly high, and clusters of dopaminergic neurons were found in the VMN [5]. There is a morphofunctional relationship between the opiate system of the brain and the noradrenergic and dopaminergic systems [5]. Hence, it can be concluded that the protease mechanism of transcriptional modulation in response to morphine operates in the SCG, blue spot, and VMN of both F344 and

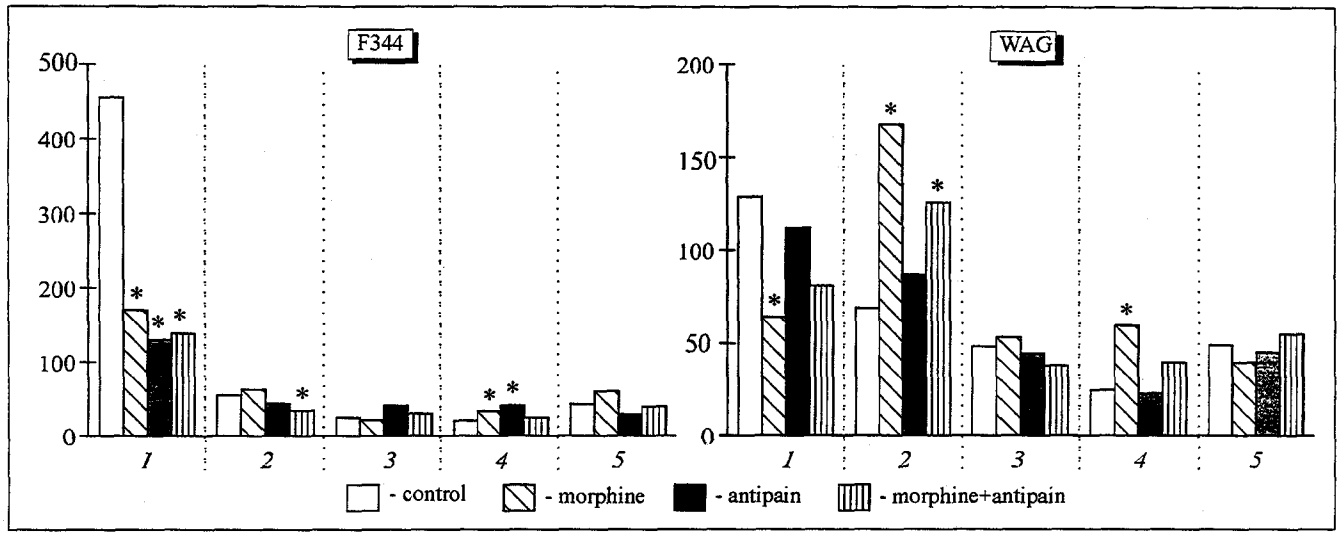


Fig. 3. Incorporation of $^3$H-uridine in total RNA of neurons and hepatocytes of 1-month-old WAG and F344 rats injected with morphine and antipain.

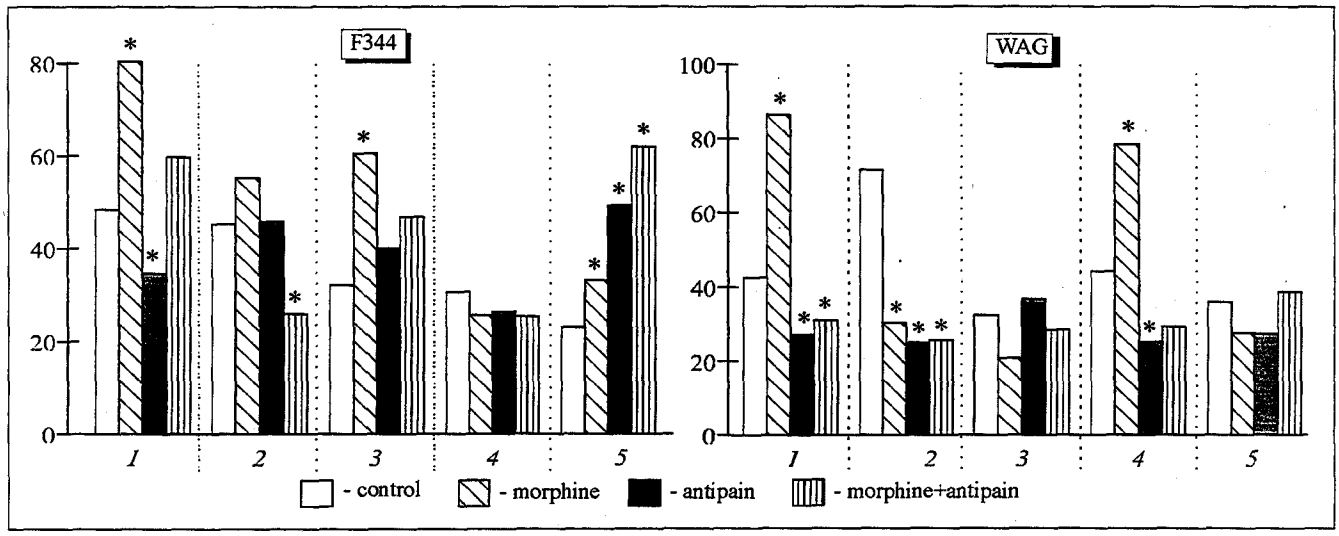


Fig. 4. Incorporation of $^3$H-uridine in total RNA of neurons and hepatocytes of 6-month-old WAG and F344 rats injected with morphine and antipain.

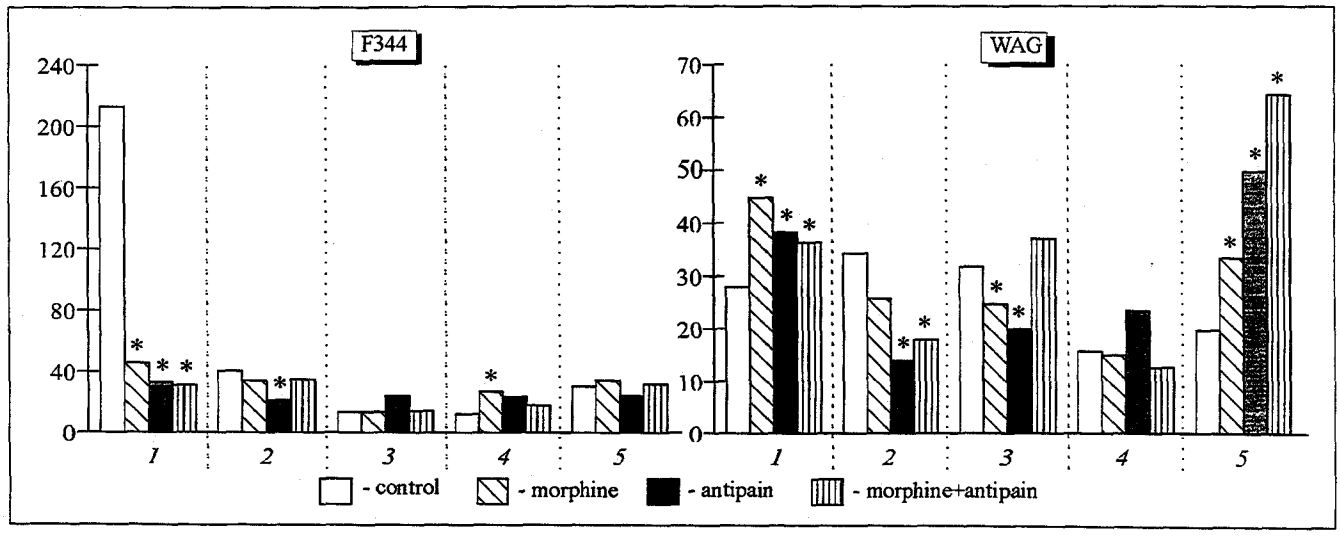


Fig. 5. Incorporation of $^3$H-uridine in total RNA of neurons and hepatocytes of 12-month-old WAG and F344 rats injected with morphine and antipain.

WAG rats. Other mechanisms of the RNA synthesis modification cannot be ruled out; they may change during postnatal ontogenesis.

Genetic differences between WGA and F344 rats manifest themselves at the levels of monoamine and endogenous opioid metabolism and opiate and adrenoreceptors [7,12]. This determines the responses of these rat strains to morphine. Morphine-induced changes in transcription in rats of different ages may be due to differentiation of some elements of the studied structures at the early stages of ontogenesis, individual genetically controlled variations in metabolism of nuclear proteins and the chromatin complex, and age-related variations in the neurotransmitter system [3,4,6,9].

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