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Intracerebral Administration of Naloxone and Drinking in Water-Deprived Rats

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SIVIY, S. M., F. BERMUDEZ-RATTONI, G. A. ROCKWOOD, C. M. DARGIE AND L. D. REID. *Intracerebral administration of naloxone and drinking in water-deprived rats*. PHARMAC. BIOCHEM. BEHAV. 15(2) 257–262, 1981.—In 24-hr water-deprived rats, naloxone, at various doses (0, 12.5, 25, 50 μg/rat), was administered prior to a 15-min drinking period. Infusions were made bilaterally into each lateral ventricle, frontal cortex, lateral preoptic area, lateral hypothalamus, and caudate nucleus. Naloxone reliably reduced water consumption at 50 μg/rat when infused into the lateral ventricles and lateral hypothalamic areas. When comparable doses of naloxone were given by peripheral injection, no effect on drinking was observed. There appeared to be a trend developing for greater sensitivity to naloxone when infusions were made into a particular part of the hypothalamus. These data support the idea that naloxone reduces drinking by acting at central opiate receptors.

Drinking Naloxone Endorphins Lateral hypothalamus Lateral ventricles

GIVEN peripherally, naloxone reduces water consumption after varying levels of deprivation and under varying conditions of testing at doses as low as 0.1 mg/kg [5, 6, 15, 16, 20, 21, 22, 25]. Naloxone decreases water intake induced by injections of either polyethylene glycol, hypertonic sodium chloride, or angiotensin II [5, 7, 22]. Doses as low as 0.0625 mg/kg of naloxone reduced consumption of 10% sucrose solutions, a solution which is quite palatable, in nondeprived rats [21,25]. No correlation was found between naloxone's ability to produce a conditioned taste aversion and its antidipsogenic effect, suggesting that the reduction in consumption is not due to a nonspecific illness [21,30]. Other opioids with antagonist properties (Win 44,441, naltrexone, diprenorphine, levallorphan, oxilorphan, and nalorphine) lead to reduced fluid intake [7, 13, 22]. In addition, it has been reported that the inactive enantiomers of opioid antagonists are ineffective in reducing water intake while the active enantiomers are effective, suggesting stereospecificity of action [7,22]. These findings lead to the hypothesis that the endogenous opioid system is more than tangentially involved in the regulation of fluid intake.

Initial studies attempting to determine if naloxone is having its antidipsogenic effect at a central or peripheral site of action have not led to a clear answer. Quaternary nalorphine, a mixed opiate agonist-antagonist which does not cross the blood-brain barrier, failed to reduce drinking induced by 24 hr of water deprivation at doses of 2, 10, or 15 mg/kg [22]. However, nalorphine, which does cross the blood-brain barrier, did reduce drinking at a dose of 10 mg/kg [22]. It was noted [22] that this finding points toward a central effect, at least for nalorphine, but it also was noted that the peripheral effects of the two compounds may not be identical. In another study [25], rats implanted with a unilateral cannula in the lateral ventricle required a dose of 100 μg of naloxone before a reliable decrease in water consumption was obtained. Since such a large amount of naloxone was required, the possibility of a peripheral effect could not be eliminated.

It is important to note that only unilateral infusions of naloxone have been done while testing for naloxone's effects on appetitive behavior. The procedure of introducing naloxone may be analogous to producing electrolytic lesions in that effects are only clearly manifest after bilateral lesions.

In the present study, bilaterally implanted cannulae were used to examine (a) whether naloxone must access both sides of the brain to be maximally effective and, more importantly, (b) to test the issue of whether naloxone's suppressive effect on water consumption can be due to a direct effect in the central nervous system.

EXPERIMENT 1

METHOD

Subjects and Apparatus

Twenty-five male, Sprague-Dawley derived (Taconic Farms) rats served as subjects. They weighed about 275 g at the time of testing. Of the 25 subjects, 15 rats were fixed with chronically indwelling bilateral cannulae. Ten subjects were used to test the effects of subcutaneously administered naloxone.

While anesthetized with pentobarbital (50 mg/kg), 15 rats were fixed with outer guide cannulae using standard stereotaxic procedures. The outer guide cannula was 23-gauge stainless steel tubing designed to accommodate a wire to block the opening or a 30-gauge inner cannula. The blocking wire extended less than 0.5 mm beyond the tip of the guide cannula into the brain. The inner cannula extended 0.5 mm beyond the tip of the guide.

There were three sites for infusion of solutions (5 rats/site): the lateral ventricles, the lateral preoptic areas, and, as a control site, the frontal cortices. The coordinates for the ventricular sites for the tips of the outer guide cannulae were 0.9 mm posterior to bregma, 1.5 mm lateral to the midline, and 4.5 mm below the top of the skull with bregma and lambda on the same horizontal plane and perpendicular to the shaft of the cannulae [19]. Using the same referents [19], coordinates for lateral preoptic area sites were 0.5, 1.5, and 8.4 mm, respectively, and cortical coordinates were bregma, 1.5, and 1.5 mm, respectively.

The rats were housed in individual cages in a room maintained at 26°C and on a light/dim light cycle (lights fully on from 2200 hr to 1000 hr). Food was always available and water was available as specified by the procedures.

Tests for drinking were conducted in the rats' home cages. Water bottles equipped with ball point sipping tubes were weighed to the nearest 0.5 g before and after a test period.

For the preoptic area and cortical sites, solutions were infused using a 30-gauge inner cannula connected to polyethylene tubing that, in turn, connected to a 5 μl syringe. The syringe plunger was driven by a Razel syringe pump at the rate of 1 μl/31 sec. For the ventricular sites, the infusions were given at the rate of 1 μl/10 sec. After each delivery of solution, the inner cannula was left in place about 1 min. All infusions were made in a 1.0 μl volume.

Procedure

Following at least 5 days to recover from surgery of fixing the cannulae, the 15 rats were placed on a drinking schedule. Every day they were given a 15 min opportunity to drink beginning from 1300 to 1400 hr. After 5 days on this schedule, a time sufficient for stabilized drinking scores, infusions were delivered 15 min before the opportunity to drink. As rats continued on the drinking schedule, they received infusions every third day. The infusions were given first to one side and then the other side with about 45 sec separating the infusions. The order of infusions (left side first or right side first) was random.

Each subject received four doses of naloxone: 0, 6.25, 12.5, and 25 μg/cannula, hence 0, 12.5, 25, and 50 μg/rat. The order of doses was counterbalanced across rats in a group (rats having the same site of infusion) so that any order effects were the same for each group.

Following this first series of tests, rats with lateral ventricular cannulae were given one other test. They were tested after a unilateral infusion of 50 μg/rat, with about one-half of the rats given the dose on the right side and the others on the left. This was to verify the finding [25] that such a dose and procedure is not sufficient to reduce drinking reliably.

The rats (n=10) without cannulae were placed on the same schedule of drinking and testing as the subjects with cannulae. They were given the same doses of naloxone 15 min before the opportunity to drink except, of course, the doses were given subcutaneously, i.e., they were given 0, 12.5, 25, and 50 μg/rat or a mean of 0, 0.04, 0.08, and 0.16 mg/kg.

Following behavioral testing, rats were sacrificed and their brains taken for histological inspection. The rats were perfused intracardially with 0.9% saline followed by a 10% Formalin solution. After the brains were removed and put into Formalin solution for a number of days, they were frozen and 80 μ slices were obtained. The slices were then treated as photographic negatives and enlarged pictures taken as an aid in determining sites of infusion.

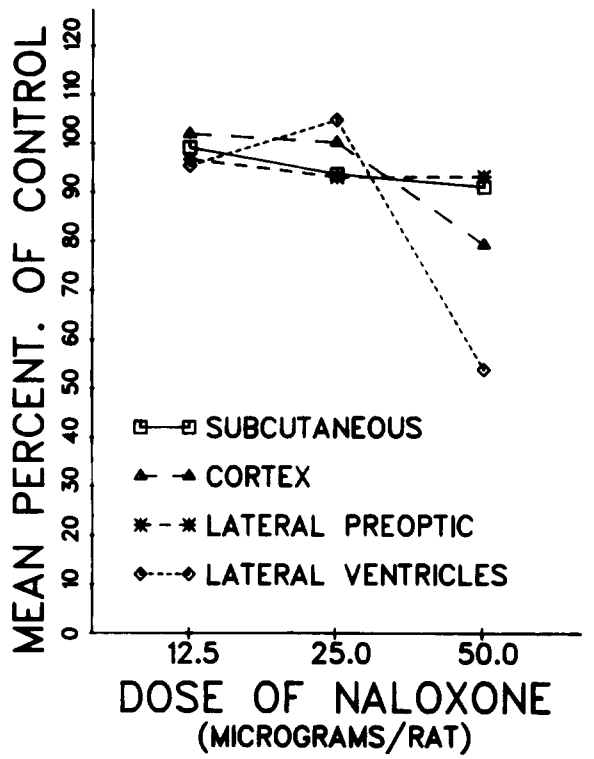

FIG. 1. The effects of various doses of naloxone on drinking in 23.75 hr water deprived rats. The values are mean percentage intake of the amount taken when given the vehicle. One group received the amounts of naloxone by way of subcutaneous injections. Three groups received the amounts of naloxone by way of two infusions intracerebrally, one half of the amount to each side. The mean percentage intake following a unilateral 50 μg infusion, which is not depicted in the figure, was 110.8% of the vehicle control. The mean amounts taken, in grams, after infusion of the vehicle for the four groups are as follows (mean g ± SEM): subcutaneous (n=10) = 17.52 ± 0.93; cortex (n=5) = 15.38 ± 1.22; lateral preoptic area (n=5) = 16.8 ± 1.58; lateral ventricles (n=5) = 15.54 ± 1.77.

RESULTS AND DISCUSSION

The dose response data are summarized in Fig. 1 which shows the mean percentages of water consumed, as compared to their vehicle control scores, by the four groups (lateral ventricular, lateral preoptic area, cortical, or peripheral administration) across the doses of naloxone. For each group's score, ANOVAs were done with the data of g of water taken/test, the data from which the percentages in Fig. 1 were derived. Only in the case of the lateral ventricular group was a significant F-value obtained, $F(3,12)=6.10$, $p<0.01$. Further analysis of the lateral ventricular group's data using the Newman-Keuls procedure showed that (a) the water consumed after a 50 μg infusion of naloxone differed from that after infusion of the vehicle ($p<0.01$), as well as after infusions of the 12.5 and 25 μg doses ($p<0.05$), and (b) water consumption after the lower two doses of naloxone (12.5 and 25 μg) did not differ reliably from the vehicle control ($p>0.05$).

The amount of water consumed after a unilateral infusion of 50 μg of naloxone into the lateral ventricle was compared to its vehicle control obtained previously by means of a *t*-test for correlated measures. The two measures did not differ reliably, mean unilateral procedure = 17.02 ± 1.77, mean of placebo procedure = 15.55 ± 1.77, $t(8)=0.59$, $p>0.05$. In summary, the only condition under which naloxone reliably decreased water consumption, in the present experiment, occurred after a bilateral 50 μg (25 μg/side) infusion into the lateral ventricles.

The enlarged photographs of the slices of the perfused brains showed that the tips of the cannulae of the lateral ventricular group were bilaterally symmetrical (in the lateral ventricles) at the level of the n. septali lateralis and about 7.5 mm anterior to the interaural plane [19]. For the preoptic area subjects, the tips of the cannulae were inside the lateral preoptic area between the (a) stria terminalis, pars commissuralis, and (b) globus pallidus, about 6.6 mm anterior to the interaural plane [19]. For the cortical group, the tips were in the frontal cortex always dorsal to the corpus callosum and 6.7 to 9.0 mm anterior from the interaural plane [19].

As can be seen from the results of this experiment, the smallest dose of naloxone infused bilaterally into the lateral ventricles that reliably reduced fluid consumption was 50 μg/rat. However, 50 μg infused unilaterally had no reliable effect. These findings confirm and extend those of a previous study [25] in which the minimally effective unilateral dose was 100 μg. Naloxone when given peripherally, at the same doses given centrally, had no discernible effect on the amount of water consumed. In addition to showing the importance of bilaterality, these results support a hypothesis of a central endorphinergic component involved in fluid intake. The preoptic area has been shown to be involved in fluid intake [2,9] and is also known to contain endogenous opioids [28]. Our failure to obtain an effect at even the highest dose of naloxone, however, leads us to suggest that the lateral preoptic area is probably not a site of opioid involvement in regulation of drinking.

EXPERIMENT 2

Results of Experiment 1 indicate that naloxone is acting centrally to depress fluid intake. To comfortably conclude that naloxone is having a central effect, however, there should be a larger difference between the minimally effective doses given peripherally and centrally. Perhaps, as the site of central injection approached a more critical area, the doses necessary to obtain a central effect would become smaller.

In this experiment, two additional sites were tested and these sites were chosen, in part, based on pilot data. The lateral hypothalamic area was examined because of its involvement in consummatory behaviors [11, 12, 26], reinforcement processes, and high concentration of endorphins [1, 28, 29]. The caudate nucleus was chosen because of its close proximity to the lateral ventricles as well as its high concentration of endorphins [1,28].

METHOD

The subjects were 21 experimentally naive, male, Sprague-Dawley derived (Taconic Farms) rats. They weighed about 350 g at the time of testing. The procedure by which the animals were fixed with bilateral cannulae was identical to that of the first experiment, except, of course, sites of the cannulae tips were in the lateral hypothalamus and caudate n. The rats were also maintained under the same environmental conditions.

There were three sets of coordinates used for the cannulae tips, one set for the caudate nucleus and two sets for lateral hypothalamic sites (7 rats/group). The coordinates for the caudate sites for the tips of the outer guide cannulae were 1.0 mm posterior to bregma, 3.0 mm lateral to the midline, and 4.5 mm below the top of the skull with bregma and lambda on the same horizontal plane and perpendicular to the shaft of the cannulae [19]. Using the same referents, coordinates for the two lateral hypothalamic areas were AP=−3.0, L=1.5, V=8.0; and AP=−3.3, L=1.5, V=8.4, respectively [19].

As in the previous experiment, all tests were conducted in the rats' home cages. Once again, a Razel syringe pump was used to deliver the infusions at the rate of 1 μl/31 sec. All infusions were made in a 1 μl volume.

The procedures were identical to those of the first experiment except that food intake was also measured. Rats were fed ad lib a powdered rat chow (Wayne Laboratory) which was kept inside their cages in food cups. When the rats were removed from their cages to be infused, their food cups were also removed and weighed to the nearest 0.5 g. After weighing the cups, they were immediately placed back in the cage. The food cups were reweighed 1 hr after the beginning of the rats' 15-min drinking period. Any spillage was collected in trays placed underneath the cage and also weighed.

RESULTS AND DISCUSSION

Results were summarized in Fig. 2. The left panel of the figure depicts drinking following infusions of doses of naloxone. For comparison, the drinking following bilateral ventricular infusions (Experiment 1) is also presented. As can be seen, 50 μg/rat to the lateral hypothalamus reduced mean percentage intakes similarly to that following bilateral ventricular infusions of the same dose. It is interesting to note that large subcutaneous doses often produce similar reductions (e.g., 10 mg/kg, SC, reducing intakes to about 70%) [15, 16, 20, 21, 25]. Statistical analyses of the data (ANOVAs followed by the Newman-Keuls method) of the hypothalamic and caudate subjects indicated that only the dose of 50 μg/rat to the lateral hypothalamus produced mean reductions that were reliably different ($p<0.05$) than control values (those following 0 μg/rat).

On the basis of inspection of enlarged photographs of brain sections, the hypothalamic subjects were grouped according to the placements of the cannulae. One group of subjects' cannulae tips were in the lateral hypothalamic zone near the fasciculus medialis prosencephali and more anterior than the other subjects' cannulae. The other group of subjects' cannulae tips were also in the lateral hypothalamic zone with the tips more posterior and probably involving the fasciculus medialis prosencephali, Forel's fields, zona incerta, and n. subthalamicus. Interestingly, this group of sites is at the anterior-posterior plane of the body of the arcuate nucleus, the site of the cell bodies of brain beta-endorphin [29].

We first ordered the subjects on the basis of anterior to posterior placement of cannulae tips. Then, we compared the drinking under naloxone of the more anterior seven subjects to that of the more posterior subjects by using a 2×4 ANOVA for repeated measures having a factor for the groups (anterior and posterior) and a factor for the four doses. The ANOVA yielded reliable group ($p<0.02$) and

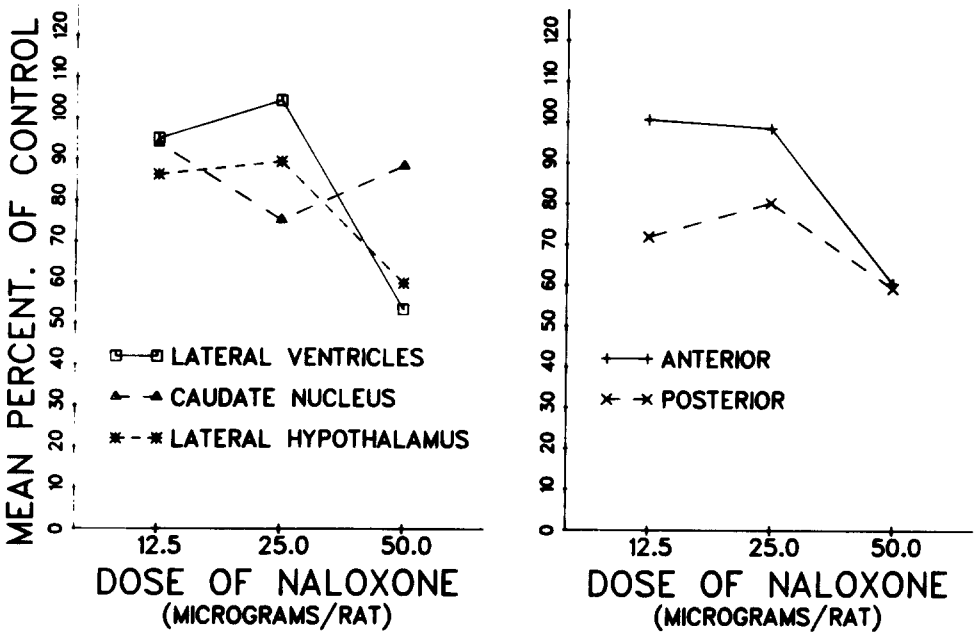

FIG. 2. The effects of bilateral infusions of various amounts of naloxone to the hypothalamus and caudate n. (left side). For comparison, scores of bilateral infusions to the ventricles (Experiment 1) are depicted. The right side depicts intakes of two groups of hypothalamic subjects, those with more anterior sites of infusions and those with more posterior sites. The mean amounts taken, in grams, after infusion of the vehicle for these groups are as follows (mean g ± SEM): caudate n. (n=7) = 16.07 ± 0.8; lateral hypothalamic area (n=14) = 14.51 ± 0.79; anterior LHA (n=7) = 15.3 ± 1.12; posterior LHA (n=7) = 13.71 ± 1.14.

dose ($p<0.02$) effects. The dose × group factor was not a reliable source of variance ($p>0.05$). The dose response relationships for these two groups can be seen in Fig. 2 (right panel). It can be seen that the more posterior group drank less at the lower two doses.

More detailed analyses comparing the posterior hypothalamic group's scores following 25 and 12.5 μg/rat failed to confirm that these smaller doses reliably reduced drinking compared to 0 μg/rat. Nevertheless, the dose-response curve does seem to be shifted with infusions into a particular part of the hypothalamus compared to the dose-response curve of bilateral ventricular infusions. The modal placements for these two groups can be seen in Fig. 3.

With respect to the amount of food eaten, statistical analyses failed to reveal any reliable effect of the intracerebral infusions of naloxone across the hour (i.e., 15 min of water availability plus 45 min following). The mean intakes with the larger infusions of naloxone to the more posterior hypothalamic area, however, were reduced compared to intakes following 0 μg/rat (mean under 0 μg = 3.5 ± 0.4 g, mean under 50 μg = 1.8 ± 0.7 g). There may be, therefore, discernible effects on feeding of intracerebral infusions under other testing conditions.

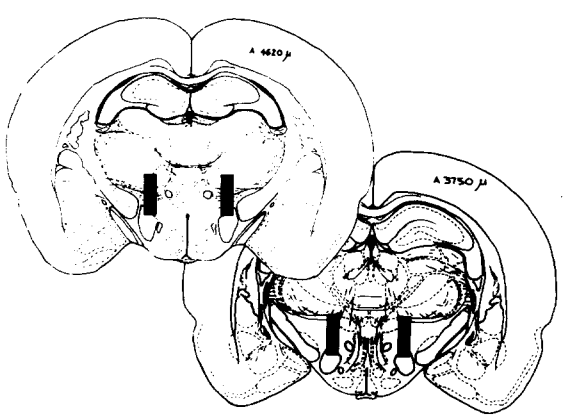

FIG. 3. A representation of the modal cannulae placements for the anterior (A 4620 μ) and posterior (A 3750 μ) lateral hypothalamic groups. The drawings were taken from the atlas of König and Klippel [19].

GENERAL DISCUSSION

Naloxone, at a dose of 50 μg given bilaterally, was effective in reducing water consumption in 24-hr deprived rats when infused into the lateral ventricles or lateral hypothalamic area. No reliable effects were obtained after infusions of this dose into either the frontal cortex, caudate n., lateral preoptic area, or when given systematically. Bilaterality appears to be a major factor as to the effectiveness of naloxone, since no attenuation was seen after unilateral infusions of 50 μg into the lateral ventricle.

As has been pointed out [24], there are problems associated with the method of intracerebral infusions of naloxone. Because of naloxone's lipophilic nature [17], it is anticipated that it will diffuse rapidly from the site of administration. Consequently, the concentrations of naloxone at the sites of infusion may be weak shortly after the infusion and the tests

reported here took place 15 min after infusions and lasted for 15 min. Considering these limitations, one can presume that only relatively large concentrations would produce an effect even when infused into a highly sensitive site. Along the same lines, naloxone administered intracerebrally may rapidly diffuse into the systemic circulation and could possibly produce its effects peripherally even though administered centrally. With the first intracerebral infusions of naloxone, while water intake was measured [25], it was found that the minimally effective dose was 100 μg/rat and that dose was nearly as large as the minimally effective peripherally administered doses. The question of whether naloxone had a unique effect when administered centrally, therefore, was left unanswered.

These results show that bilateral infusions are more effective than unilateral infusions (i.e., smaller doses produce reliable reductions in drinking when administered bilaterally). When bilateral infusions were made, the minimally effective intracerebral dose will not produce a reliable effect when administered peripherally (Experiment 1). These data support the idea that naloxone reduces drinking by acting centrally. More importantly, no effect of naloxone was discernible when administered into either the frontal cortices, caudate n., or lateral preoptic areas, at the doses tested. Since the infused naloxone can easily access the systemic circulation from any one of these sites, it does not appear that centrally administered naloxone, at the doses tested in this report, is producing its effect at a peripheral site of action.

It has been suggested [5], since pituitary factors seem minimally involved in the naloxone-effect on drinking, that the hypothalamic area is a likely site of action. Our data, although not clearly confirming that idea, since only limited sites were sampled, do support the idea that some tissue near or in the lateral hypothalamic area is particularly sensitive to naloxone. For example, zona incerta lesions do produce behaviors similar to those produced by naloxone, i.e., these lesions reduced daily intakes and established refractoriness with respect to hypertonic stimuli to drink [23]. Our most posterior sites, however, were our most effective sites and, therefore, there is a need to explore other posterior sites. An interesting speculation follows from our initial explorations of CNS effects with naloxone. Our most sensitive sites were relatively near the arcuate nucleus, which contains the single major cell group of brain beta-endorphin [29]. In rats, beta-endorphin increases consumption of liquid food [14,18]. In human narcotic addicts, infusions of beta-endorphin brings about a feeling of thirst [27]. However, met- and leu-enkephalin have been shown to inhibit water intake in rats [8,10]. Therefore, it follows that naloxone may be producing its antidipsogenic effect by acting at beta-endorphin cell bodies within the arcuate nucleus. However, for such an explanation to be plausible it must first be demonstrated that autoreceptors are present on these cell bodies. The fields of innervation of the arcuate nucleus fibers should also be examined in future studies. Finally, our most effective cannulae placements were also near the ventral tegmental area, an area shown to be highly involved in the rewarding effects of opiates [3,4].

Having demonstrated, with these experiments, and others [22,25], the possibility of opioid antagonists having central nervous system effects that lead to suppressed drinking, the next steps are to work out the methods for a systematic search for the tissue that is particularly sensitive to antagonist effects.

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