Non-Steroidal Anti-Inflammatory Drugs Potentiate the Vasoconstrictor Effects of Ouabain in the Dog

ALAN S. NIES AND JOHN G. GERBER

SUMMARY The purpose of this study was to determine whether inhibitors of prostaglandin synthesis alter the peripheral vascular effects of the cardiac glycoside, ouabain. Ouabain (35 /µg/kg) was given to 12 mongrel dogs, six of which had been pretreated 1 hour previously with the non-steroidal anti-inflammatory drugs (NSAID), indomethacin or meclofenamate (6 mg/kg, iv, bolus followed by 1 mg/min infusion). Hemodynamic variables were monitored for 30 minutes before and for 3 hours after ouabain administration. Mean arterial pressure was significantly increased in all dogs for 45 minutes after ouabain, and cardiac output was unchanged. In the animals that did not receive non-steroidal anti-inflammatory drugs, ouabain transiently (15 min) decreased renal blood flow and increased renal vascular resistance. Mesenteric blood flow was not altered by ouabain until 90 minutes, after which time it was significantly increased. In the dogs pretreated with NSAID, renal blood flow was reduced for 60 minutes after administration of ouabain. In addition, 30 minutes after ouabain, the mesenteric blood flow was reduced by 43 ± 17 ml/min from a baseline value of 192 ± 42 ml/min (P < 0.05), and this reduction persisted for the entire 3-hour observation period. Control animals that did not receive ouabain showed neither an increase in arterial pressure nor a decrease in renal or mesenteric blood flow over the period of the experiment. Thus, pretreatment with NSAID enhanced the duration of the renal vasoconstrictor effects of ouabain in the dog and converted late mesenteric vasodilation to early and persistent vasoconstriction. These data imply a potentially important role for vasodilating prostaglandins in modulating the mesenteric as well as the renal circulatory responses to ouabain.


DIGITALIS glycosides not only have a positive inotropic effect on the myocardium, but also produce constriction of the peripheral vasculature (Ross et al. 1960; Braunwald et al., 1961; Mason and Braunwald, 1964; Walhausen and Herendeen, 1964; Harrison et al., 1969; Treat et al. 1971). This extracardiac effect of the cardiac glycosides results in an increase in vascular resistance of several regional vascular beds in humans and in experimental animals. Although usually of no clinical consequence, a digitalis-induced increase in mesenteric vascular resistance has been postulated to be important in the pathogenesis of non-occlusive intestinal ischemia seen in debilitated patients with congestive heart failure (Shanbour and Jacobsen, 1972; Levinsky et al. 1975; Lanciault and Jacobsen, 1976).

Since some of the prostaglandins are potent dilators of the intestinal circulation (Dusting et al. 1978) and since the intestinal vasculature has the capacity to produce vasodilatory prostaglandins (Bunting et al. 1976), we postulated that local prostaglandin production by the gastrointestinal circulation might be one mechanism by which drug-induced intestinal vasoconstriction is modulated. If this hypothesis were true, the gastrointestinal circulation would be similar to the renal circulation where production of vasodilator prostaglandins diminishes whereas inhibition of prostaglandin synthesis enhances the effects of vasoconstrictor stimuli (Aiken and Vane, 1973; Swain et al., 1975). We tested this hypothesis with ouabain and nonsteroidal anti-inflammatory drugs (NSAID) in anesthetized dogs and compared the responses of the superior mesenteric and renal circulations.

Methods

Twelve mongrel dogs of either sex weighing 17-29 kg were anesthetized with sodium pentobarbital (25 mg/kg, iv) and ventilated with room air through an endotracheal tube. Supplemental doses of pentobarbital were given as needed to maintain anesthesia. Polyethylene catheters were placed in the femoral artery and in the right atrium via the femoral vein. Through a midline abdominal incision, non-cannulating electromagnetic flow probes (Gould/Statham SP7515) were placed around the superior mesenteric artery and one renal artery.

After a 30-minute stabilization period, the dogs were assigned to one of two groups. One group of six dogs received either indomethacin (Sigma Chemical Co.; three dogs) or meclofenamate (a gift from Parke-Davis & Co.; three dogs) as a 6 mg/kg
Peripheral resistance was calculated by dividing the mean arterial pressure in mm Hg by the cardiac output (liters/min). Mesenteric and renal vascular resistances were calculated by dividing the mean arterial pressure (mm Hg) by the organ blood flow (ml/min) without correcting for portal or systemic venous pressure.

To control for the effects of time and deterioration of the experimental preparation, we studied two additional groups of dogs that did not receive ouabain. One group (n = 5) was pretreated with NSAID (two with meclofenamate; three with indomethacin). The other group (n = 5) was pretreated with sodium carbonate solution. All dogs were followed for the same period of time as the dogs receiving ouabain.

In another four dogs, a catheter was placed into the superior mesenteric branch of the portal vein, and 30-ml blood samples were obtained before and 15 minutes after ouabain administration (35 μg/kg, iv) for determination of the concentration of 6-keto prostaglandin F₁₅, the hydrolysis product of prostaglandin I₂. The blood samples were drawn into chilled syringes containing 3 ml of 1 mm indomethacin and 3.8% sodium citrate. After addition of tetradecuter 6-keto PGF₁₀ as an internal standard and a tracer amount of tritiated 6-keto PGF₁₀, the samples were extracted at pH 3 with ethyl acetate. The organic extract was partially purified with an open silicic acid column and further purified by reversed phase high performance liquid chromatography. The 6-keto PGF₁₀ was derivatized to the methyl ester, methoxime, trimethyl silyl ether derivative for injection into the gas chromatograph-mass spectrometer (GC-MS). Quantification was accomplished by monitoring the ion pairs m/e 598 and 602 corresponding to the unknown and internal standard, respectively.

Statistics
The significance of the effects of ouabain or time in each group of dogs was determined by an analysis of variance with a Newman-Keuls post hoc test. The significance of the effects of time and deterioration of the experimental preparation was determined by a two-way ANOVA.

Table 1: The Effect of Ouabain on Systemic Hemodynamics

<table>
<thead>
<tr>
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<th>Effects at</th>
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<tbody>
<tr>
<td></td>
<td>-30 min</td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac output</td>
<td>2.69 ± 0.18</td>
</tr>
<tr>
<td>(liters/min)</td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>143 ± 8</td>
</tr>
<tr>
<td>Peripheral resistance (mm Hg/liter per min)</td>
<td>54.2 ± 4.7</td>
</tr>
</tbody>
</table>

NSAID-pretreated animals

<table>
<thead>
<tr>
<th></th>
<th>Effects at</th>
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<tbody>
<tr>
<td></td>
<td>-30 min</td>
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<td></td>
<td></td>
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<tr>
<td>Cardiac output</td>
<td>1.89 ± 0.22</td>
</tr>
<tr>
<td>(liters/min)</td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>152 ± 3</td>
</tr>
<tr>
<td>Peripheral resistance (mm Hg/liter per min)</td>
<td>85.9 ± 9.4</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE. Ouabain (35 μg/kg) was given at time 0.
* P < 0.05 compared with baseline values (Dunnett's test).
of variance using Dunnett’s method of multiple comparison with a control. The changes produced by ouabain with and without the NSAID were compared at each time point with Student’s t-test for independent groups.

Results

All hemodynamic variables were stable during the 30 minutes prior to ouabain administration as indicated by the -30-minute and -15-minute time points on the tables and figures. In the absence of NSAID, ouabain produced a rise in mean arterial pressure within 15 minutes and pressure remained significantly elevated for 45 minutes (Table 1). Cardiac output was not significantly affected by ouabain. Renal blood flow fell and renal vascular resistance rose after ouabain but were significantly changed only at the 15-minute time point, after which they returned toward baseline (Table 2). Superior mesenteric blood flow and mesenteric vascular resistance were not significantly altered within the first hour after ouabain but, at 90 minutes and thereafter, mesenteric blood flow was increased.

In the animals pretreated with NSAID, the baseline values for the hemodynamic parameters were not significantly different from the control animals, although cardiac output and regional blood flows tended to be lower and systemic and regional vascular resistances tended to be higher (Tables 1 and 2). Ouabain administration to the NSAID-treated animals produced an increase in mean arterial pressure comparable to that seen in the control animals and was associated with a rise in peripheral resistance. Renal blood flow was significantly decreased and renal vascular resistance significantly increased.

### Table 2 The Effect of Ouabain on Regional Hemodynamics

<table>
<thead>
<tr>
<th>Effects at</th>
<th>-30 min</th>
<th>-15 min</th>
<th>0 min</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal blood flow (ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle-pretreated animals</td>
<td>203±32</td>
<td>204±34</td>
<td>203±30</td>
<td>176±28*</td>
<td>189±24</td>
<td>184±27</td>
<td>181±25</td>
<td>203±27</td>
<td>213±25</td>
<td>230±28</td>
</tr>
<tr>
<td>Renal vascular resistance (mm Hg/ml per min)</td>
<td>0.76±0.08</td>
<td>0.76±0.09</td>
<td>0.76±0.18*</td>
<td>0.92±0.09</td>
<td>0.97±0.13</td>
<td>0.89±0.08</td>
<td>0.74±0.05</td>
<td>0.67±0.05</td>
<td>0.62±0.06</td>
<td></td>
</tr>
<tr>
<td>Mesenteric blood flow (ml/min)</td>
<td>230±35</td>
<td>221±31</td>
<td>220±32</td>
<td>212±32</td>
<td>253±26</td>
<td>262±19</td>
<td>267±16</td>
<td>278±33*</td>
<td>289±50*</td>
<td>286±45*</td>
</tr>
<tr>
<td>Mesenteric vascular resistance (mm Hg/ml per min)</td>
<td>0.74±0.17</td>
<td>0.74±0.14</td>
<td>0.75±0.15</td>
<td>0.75±0.37</td>
<td>0.72±0.13</td>
<td>0.65±0.08</td>
<td>0.58±0.06</td>
<td>0.57±0.09</td>
<td>0.568±0.14</td>
<td>0.57±0.13</td>
</tr>
<tr>
<td>NSAID-pretreated animals</td>
<td>149±13</td>
<td>152±15</td>
<td>153±16</td>
<td>127±18*</td>
<td>119±12*</td>
<td>113±13*</td>
<td>115±15*</td>
<td>138±20</td>
<td>140±21</td>
<td>137±20</td>
</tr>
<tr>
<td>Renal blood flow (ml/min)</td>
<td>1.06±0.27</td>
<td>1.08±0.14</td>
<td>1.08±0.16</td>
<td>1.54±0.18*</td>
<td>1.67±0.17*</td>
<td>1.69±0.20*</td>
<td>1.61±0.25*</td>
<td>1.34±0.25</td>
<td>1.28±0.22</td>
<td></td>
</tr>
<tr>
<td>Renal vascular resistance (mm Hg/ml per min)</td>
<td>190±40</td>
<td>195±40</td>
<td>192±40</td>
<td>158±31*</td>
<td>149±28*</td>
<td>143±27*</td>
<td>135±34*</td>
<td>144±31*</td>
<td>140±26*</td>
<td></td>
</tr>
<tr>
<td>Mesenteric blood flow (ml/min)</td>
<td>158±40</td>
<td>192±40</td>
<td>158±40</td>
<td>149±31*</td>
<td>143±28*</td>
<td>135±27*</td>
<td>144±34*</td>
<td>140±31*</td>
<td>145±26*</td>
<td></td>
</tr>
<tr>
<td>Mesenteric vascular resistance (mm Hg/ml per min)</td>
<td>1.00±0.20</td>
<td>1.02±0.22</td>
<td>1.00±0.20</td>
<td>2.11±0.98*</td>
<td>1.71±0.52*</td>
<td>1.59±0.38*</td>
<td>1.64±0.41*</td>
<td>1.61±0.44*</td>
<td>1.48±0.36*</td>
<td>1.20±0.25</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE. Ouabain (35 μg/kg) was given at time 0.

* P < 0.05 compared with 0 time values (Dunnett’s test).
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for an hour after ouabain administration in the NSAID group. Mesenteric blood flow was decreased significantly at 45 minutes and was decreased in five of the six animals by 15 minutes. The decrease in mesenteric blood flow persisted for the entire 3 hours of observation. Similarly, mesenteric vascular resistance was increased within 15 minutes and remained significantly elevated for 2 hours.

The influence of pretreatment with the NSAID on the renal and mesenteric vascular responses to ouabain can be appreciated in Figures 1 and 2 where the changes from baseline produced by ouabain in the presence or absence of NSAID are compared, and the significance of the differences between the two groups is indicated. The ouabain-induced changes in renal blood flow were not significantly different in the two groups, but there was a significantly greater influence in the renal vascular resistance at 30, 45, and 60 minutes in the animals pretreated with NSAID (Fig. 1). The response of the superior mesenteric vasculature to ouabain was markedly different in the two groups of animals (Fig. 2). The dogs that had received the NSAID pretreatment had a significantly greater rise in mesenteric vascular resistance beyond the 30-minute observation period. The changes in mesenteric blood flow were in opposite directions in the two groups, being decreased by ouabain in the NSAID group, and significant differences in the response to ouabain were seen at 30 minutes and persisted for the remainder of the 3-hour observation period.

The fact that the significant changes produced by ouabain are not related to changes in the experimental model with time is indicated by the results in Table 3. In the absence of ouabain, there were no significant increases in arterial pressure or decreases in renal or mesenteric blood flows. The only significant change observed was a gradual increase in renal blood flow with time in the vehicle-pretreated animals.

Analysis of all the portal plasma samples by GC-MS indicated that all samples contained < 200 pg/ml 6-keto PGF₁α, which was the limit of our assay.

Discussion

The cardiac glycosides are known to produce vasoconstriction directly and possibly through activation of the adrenergic nervous system (Ross et al., 1960; Stark et al., 1972; Levinsky et al., 1975; Treat et al., 1971). This effect is not specific for any vascular bed. However, the duration and magnitude of the vasoconstriction produced depends on the experimental preparation. Harrison et al. (1969), used anesthetized dogs with an exteriorized loop of small intestine and found mesenteric vascular resistance to be increased for 30 minutes following an intravenous injection of 50 μg/kg ouabain, although mesenteric blood flow was not altered consistently. Pawlik and Jacobsen (1974) showed that blood flow to an isolated segment of the canine intestine was decreased within 10 minutes by digoxin (5 μg/kg), but by 50 minutes the flow had returned to baseline. Treat et al. (1971) found that ouabain infused into the mesenteric artery at rates of 5–80 μg/min produced transient reductions in mesenteric blood flow that disappeared when the infusion was stopped. Higgins et al. (1972), used unanesthetized dogs and found ouabain (20 μg/kg, iv) to increase mesenteric vascular resistance for less than 5 minutes, and, at
15 and 30 minutes, the mesenteric vascular resistance was actually decreased and mesenteric blood flow increased. These investigators found renal vascular resistance to be increased for the entire 30-minute period and renal blood flow to be depressed for this time. Finally, in the anesthetized rhesus monkey, Shanbour et al. (1971) found that injection of ouabain (20 to 35 µg/kg, iv) was followed by a late fall in mesenteric blood flow, but no untreated controls were studied for comparison.

Our data are in general agreement with the published observations to the effect that cardiac glycosides increase vascular resistance but that these effects are not persistent. The increase in vascular resistance was seen in our experiments as an early increase in arterial pressure. However, in our model, any effect of ouabain to constrict the mesenteric vascular bed must have been early and short-lived since, by 15 minutes, mesenteric blood flow was not significantly changed from baseline and, at later times, mesenteric blood flow increased. These findings agree with the observations of Higgins et al. (1972). In the other cited reports of the effects of ouabain on the canine mesenteric vasculature, observations were not made for more than 30 minutes so that persistence of the effects was not examined. In addition, data from intra-arterial infusions or from animals with isolated intestinal segments may not be entirely comparable to ours.

Pretreatment with the NSAID, indomethacin or meclofenamate, caused the vasculature to respond to ouabain in quite a different manner. The mesenteric vascular resistance rose and mesenteric blood flow fell, and these effects were sustained for up to 3 hours, in contrast to the effects of ouabain alone. Additionally, the ouabain-induced rise in renal vascular resistance was greater in the NSAID-pretreated than in the vehicle-pretreated dogs, although this difference was maintained for only 1 hour. In those that did not receive ouabain, there was no decrease in mesenteric or renal blood flow over the 3-hour period, indicating that the effects observed after ouabain were, in fact, induced by ouabain.

Both indomethacin and meclofenamate are potent inhibitors of prostaglandin synthesis, but they differ in other ways (Flower, 1974). We used both drugs so that our findings could be generalized to other NSAID. Since the responses to ouabain were altered in a similar manner by both drugs, the data were combined into a single NSAID group for statistical comparison with control.

The renal vascular bed of the dog has been shown to have an enhanced vasoconstrictor response to angiotensin II and α-adrenergic agonists when prostaglandin synthesis is inhibited, implying a role for renal prostaglandins in modulating the vascular effect of these vasoconstrictors (Aiken and Vane, 1973; Swain et al., 1975). Our data indicate that these observations extend to the renal vasoconstric- tor effects of ouabain. The role of prostaglandins in the vasculature of the gastrointestinal tract is unknown. Basal and stimulated gastric blood flow is decreased by NSAID (Gerkens et al., 1977). Mesenteric blood flow can be increased with prostaglandin E2 and I2, and indomethacin decreases intestinal blood flow during pentagastrin stimulation (Dusting et al., 1978; Gerkens et al., 1977). The fact that
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NSAID converted the ouabain-induced increase in mesenteric blood flow to a profound and sustained decrease in blood flow and rise in mesenteric vascular resistance implies an important role for prostaglandins in the intestine.

In spite of the fact that we could not detect 6-keto PGF₁α in the portal venous blood, the best explanation for our data is that a vasoconstrictor prostaglandin is released and modulates the vascular effects of ouabain. PG₁₂ could have been present in a concentration less than 200 pg/ml, or another vasodilator such as PGE₂ could have been released. An alternate explanation is that the NSAID enhanced ouabain-induced vasoconstriction by a mechanism unrelated to the prostaglandin system. We feel this explanation is less likely because the two structurally dissimilar prostaglandin synthesis inhibitors gave similar results.

The mechanism whereby ouabain might stimulate prostaglandin synthesis is unknown. Cardiac glycosides are known to inhibit the action of the membrane sodium-potassium ATPase. Many investigators have postulated that this effect results in an increase in available intracellular calcium which is responsible for both the inotropic and vasoconstricting effects of the cardiac glycosides (Schwartz et al., 1975; Belardinelli et al. 1979). It is also likely that calcium is the key to initiating the events leading to prostaglandin production. The initial step in prostaglandin synthesis is the liberation of arachidonic acid from an esterified form, and phospholipase A₂ is a calcium-activated enzyme that is thought to catalyze this initial step (Cheung, 1980). In platelets and in renal papillary tissue in vitro, A₂₃₁₈₇, a calcium ionophore, is a potent stimulus of prostaglandin production (Oelz et al. 1978), and ouabain has been shown recently to increase prostaglandin production in the renal papillae in vitro (Knapp and Oates, 1980). It can be hypothesized that, in the vascular system, ouabain results in an increase in intracellular calcium that is available not only to the contractile proteins but, also, to activate enzymes responsible for initiating prostaglandin synthesis. Blockade of vasoconstrictor prostaglandin synthesis by NSAID leaves the vasoconstricting influences unopposed and, hence, the increase in vascular resistance produced by the ouabain is enhanced.

The mesenteric vasoconstriction produced by the cardiac glycosides has been postulated to be responsible for some instances of non-occlusive ischemic necrosis of the intestine. From our data, it would seem unlikely that cardiac glycoside alone could be responsible for this condition, since the effects of ouabain to produce mesenteric vasoconstriction are small and transient, and heart failure has been shown to diminish any mesenteric vasoconstriction produced by ouabain (Higgins et al., 1972). However, in the presence of NSAID, ouabain produced profound mesenteric vasoconstriction that persisted for at least 3 hours. Since NSAID are among the most commonly used over-the-counter and prescription drugs, it is likely that the combined use of cardiac glycosides and NSAID is not an uncommon occurrence. It is possible, therefore, that the drug interaction demonstrated by our data in the dog could have clinical relevance.

References

The Utilization by Rabbit Aorta of Carbohydrates, Fatty Acids, Ketone Bodies, and Amino Acids as Substrates for Energy Production

KENNETH V. CHACE AND RICHARD ODESSEY

SUMMARY

The ability of rabbit aorta to oxidize various substrates was studied to determine which of these compounds may be energy substrates for vascular smooth muscle (VSM). Glucose, ketone bodies, medium-chain length fatty acids, branched-chain amino acids, and glutamine all are oxidized at comparable rates on a molar basis. Some other amino acids, long chain fatty acids, pyruvate and glycerol also are oxidized, but at lower rates. The oxidation of 8 amino acids could not be detected. VSM was found to release ketone bodies when incubated in leucine/$\beta$-hydroxybutyrate or octanoate. This suggests that the acetoacetyl CoA and/or acetoacetate derived from these substrates is not completely oxidized. The oxidation rate of several substrates when measured individually is inhibited by 50–80% by the presence of a combination of other substrates in the medium. Under these conditions, glucose is a minor substrate for oxidative metabolism accounting for only 5% of O$_2$ consumption. The oxidation rate of all the exogenous substrates together is calculated to account for less than half of the oxygen consumption; this finding indicates that an endogenous substrate must also be utilized. Circ Res 48: 850–858, 1981

SEVERAL investigators have examined the metabolism of vascular smooth muscle (VSM) by studying the effect of stimulation (Lundholm and Mohme-Lundholm, 1962; Peterson and Paul, 1974) or the effect of age or disease (Morrison et al., 1972b; Daly, 1976) on its metabolism. They have generally assumed that glucose is the major energy source for VSM, and have not attempted to examine the metabolism of other possible energy sources.

One reason for this assumption is that VSM has a respiratory quotient of 0.99 (Kirk et al., 1954, Kosan and Burton, 1966). However, the studies that established this figure were performed with 11 mm glucose as the only substrate. Since VSM can oxidize fatty acids (Hashimoto and Dayton, 1971; Morrison, et al., 1974) and amino acids (Morrison et al., 1976b), it is quite possible that incubation of VSM in a physiological mixture of all possible energy substrates present in the blood would reduce the respiratory quotient.

Glucose also was thought to be the major energy source for VSM because carbohydrate was more effective than other substrates in restoring contractility to substrate-depleted tissue (Coe et al., 1968). In this study, it was assumed that tissue could not contract after incubation without substrate because the high-energy phosphates in the tissue were exhausted. Measurements were not made to determine whether the inability of substrate-depleted tissue to contract was due to its low-energy state, or whether substrates that restored its ability to contract improved its energy state. Since the restoration of the ability of VSM to contract could be caused by means other than the regeneration of high-energy phosphate, (Edwards et al., 1977; Roberts et al., 1979, Pittman and Quinn, 1979), this study did not really determine what substrates are most effective at generating high-energy phosphate.

The question of which substrates are most effec-
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A S Nies and J G Gerber

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