Direct Effect of Beta-Adrenergic Stimulation on Renin Release by the Rat Kidney Slice In Vitro

By Myron H. Weinberger, Wataru Aoi, and David P. Henry

ABSTRACT

Controversy exists regarding the mechanism by which catecholamines stimulate renin secretion in vivo. A sensitive rat kidney slice system was utilized to study the direct effects of adrenergic agonists and antagonists on renin release in vitro. Catecholamines were protected from degradation by the addition of ascorbic acid to the incubation medium. Significant dose-related stimulation of renin release was observed with epinephrine and norepinephrine in concentrations from $1.5 \times 10^{-8}$ to $1.5 \times 10^{-7}$M and with isoproterenol in concentrations from $2 \times 10^{-9}$ to $2 \times 10^{-7}$M. No significant stimulation was seen with $10^{-6}$M concentrations of the three agents. Methoxamine ($10^{-6}$M) stimulated renin release significantly ($P < 0.01$). The stimulation observed with epinephrine, norepinephrine, or isoproterenol was blocked by $d,l$- and $Z$-propranolol ($2 \times 10^{-4}$M) but not by $d$-propranolol ($2 \times 10^{-4}$M) or phentolamine ($9 \times 10^{-4}$M). Methoxamine-induced stimulation was abolished by $d,l$-propranolol but not by phentolamine. These data indicate that the in vitro kidney slice system is responsive to physiological concentrations of catecholamines when they are protected from degradation. The results further demonstrate a direct stimulatory role for $\beta$-adrenergic agents on renin release and suggest that $\alpha$-adrenergic effects seen in vivo are mediated indirectly by hemodynamic, vascular, or functional changes in the kidney.

Although the physiological role of the sympathetic nervous system in the regulation of renin secretion in vivo has been demonstrated in numerous studies (1–3), the direct effect of adrenergic agents on the renin-releasing cells is not completely understood. In the intact animal, stimulation of the renal nerves (4–8) and infusion of catecholamines into the renal artery (4, 6, 9) increase renin release. Conflicting evidence concerning the mediation of catecholamine stimulation of renin release is apparent from studies of intact animals and man. A major role for $\beta$-adrenergic stimulation of renin release has been established (1, 5, 7, 8, 10–12). Other studies have suggested that $\alpha$-receptors are also involved (8, 10, 11). The interpretation of such observations in the intact system is rendered difficult in view of the variety of influences on hemodynamics, circulating humoral factors, and renal tubular function exerted by the agents utilized in such studies.

A consistent, sensitive and specific method for the study of renin release by rat kidney slices in vitro has permitted the evaluation of direct effects of isolated stimuli (13, 14). Previous studies utilizing such systems have demonstrated stimulation of renin release by pharmacologic concentrations of catecholamines (14–17). However, such in vitro studies have not demonstrated an effect of catecholamines on renin release in the concentrations known to exist in the circulation (18). Thus, the significance of these previous studies is not clear.

The present study was designed to examine the direct effects of adrenergic agents, in physiological concentrations, on an isolated kidney slice system. To investigate the specificity of catecholamine-induced stimulation of renin release, the effects of the adrenergic agonists, norepinephrine, epinephrine, isoproterenol, and methoxamine, and of the adrenergic antagonists, phentolamine and propranolol, were studied.

Methods

KIDNEY SLICES

Male Sprague-Dawley rats weighing 180–250 g were maintained on a regular diet. Kidney slices were prepared and incubated by a previously described method (14). In brief, both kidneys were removed under sodium pentobarbital anesthesia and placed in ice-cold saline. Two 10–20-mg (dry weight) slices (0.5 mm in thickness) were prepared from each kidney using a Stadie-Riggs microtome; these slices were incubated in 2.0 ml of Robinson's medium containing 5.65 mM glucose at 25°C in a shaking incubator saturated with 95% O₂-5% CO₂. The pH of the medium was 7.4 and the osmolarity was maintained at 350 mosmoles/liter by the addition of

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This study was supported by U. S. Public Health Service Grants HL 13764 and HL 14159 from the National Heart and Lung Institute and by a grant-in-aid from the American Heart Association, Indiana Affiliate.

Received February 6, 1975. Accepted for publication June 30, 1975.
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manitol. Previous studies (14) have demonstrated osmolar stimulation of renin release. For this reason, the osmolarity of the medium was adjusted to a constant 350 mosmoles/liter throughout all control and experimental periods to permit the addition of the experimental agents in various concentrations. Each slice was preincubated for 15 minutes. The preincubation medium was then aspirated, and the slice was incubated with 2.0 ml of fresh medium for four successive 20-minute periods (A-D). The incubation medium was aspirated after each period and added to a tube containing 20 µliters of dimercaprol (100 mg/ml), 20 µliters of 8-hydroxyquinoline (0.34 M), and 0.1 ml of 4.0% ethylenediaminetetra-acetic acid (EDTA). These agents inhibit both angiotensinase and converting enzyme.

EXPERIMENTAL STUDIES

Since catecholamines are rapidly degraded at alkaline pH (19), a stabilizing substance, ascorbic acid (6 x 10^{-3}M), was added. The effect of ascorbic acid on renin release was evaluated by adding it during the experimental period and comparing renin release with that during the control period in which the medium was devoid of ascorbic acid. Norepinephrine bitartrate (Calbiochem) was added to the medium in concentrations varying from 1.5 x 10^{-1} to 1.5 x 10^{-4}M. Epinephrine bitartrate (Calbiochem) and isoproterenol hydrochloride (Sigma) were added to the medium in similar concentrations. In addition, studies were performed utilizing 10^{-3}M methoxamine (Vasoxyl, Burroughs-Wellcome).

The effect of adrenergic blocking agents on renin release was evaluated by adding the agent during the experimental period and comparing renin release with that during the control period in which the medium was devoid of the agent. Then, the effect of adrenergic blocking agents on the catecholamine-induced stimulation of renin release was examined by adding the blocking agent to the medium in the preincubation period and the four successive 20-minute incubation periods. The catecholamine was then added in the third incubation period (C). The effects of epinephrine, norepinephrine, and isoproterenol on renin release were studied in the presence of 9 x 10^{-1}M phenolamine (CIBA) or 2 x 10^{-4}M d, l- or d, l-propranolol (Ayerst). In addition, the effects of 2 x 10^{-7} and 2 x 10^{-8}M d, l-propranolol on isoproterenol-induced renin release were examined. The effect of 10^{-3}M methoxamine was studied in the presence of similar concentrations of phenolamine and d, l-propranolol. The effect of isoproterenol (2 x 10^{-4}M) was studied by adding the agent in the third incubation period (C) to slices that had been incubated with medium containing methoxamine (10^{-5}M) for the entire experiment.

ANGIOTENSIN I GENERATION AND QUANTIFICATION OF RENIN RELEASE

Renin-containing medium (0.1 ml) from the kidney slice incubate was added to 1.25 nm hog renin substrate (Pentex) with angiotensinase and converting enzyme inhibitors and incubated for 1 hour at 37°C in a shaking incubator (14). The amount of angiotensin I thus generated per milligram dry kidney weight. The substrate blank yielded a mean value ± SE of 0.90 ± 0.08 ng/mg (N = 28). The influence of the adrenergic agonist or antagonist on angiotensin generation was evaluated by using a blank containing the agent, medium, and substrate. In no experiment did the agonists or antagonists used increase angiotensin I generation from substrate when the incubation was in renin-free medium. The agents used had negligible effects directly on the radioimmunoassay of angiotensin I when they were added alone or on the generation of angiotensin I from substrate as shown in Table 1.

The statistical significance of the experimental observations was evaluated using a paired t-test (21) comparing the experimental period to the preceding control period. Previous studies utilizing this technique have demonstrated the stability of basal renin release over four consecutive 20-minute incubation periods, thus permitting each kidney slice to serve as its own control for experimental studies (14).

Results

EFFECT OF ASCORBIC ACID AS A STABILIZING FACTOR OF CATECHOLAMINES

Since catecholamines are not stable at alkaline pH (19), ascorbic acid (6.0 x 10^{-3}M) was added to the Robinson’s medium as an antioxidant. Figure 1 demonstrates the effect of ascorbic acid in enhanc-

<table>
<thead>
<tr>
<th>Agent</th>
<th>N</th>
<th>Apparent angiotensin I (ng/0.05 ml)</th>
<th>Angiotensin I generation with added substrate (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate</td>
<td>28</td>
<td>0.30 ± 0.02</td>
<td>28 0.90 ± 0.08</td>
</tr>
<tr>
<td>Rat renin</td>
<td>3</td>
<td>0.51 ± 0.03</td>
<td>2 1.06</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>19</td>
<td>0.39 ± 0.03</td>
<td>2 1.02</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>4</td>
<td>0.55 ± 0.02</td>
<td>2 1.02</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>2</td>
<td>0.44</td>
<td>3 1.19 ± 0.26</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>4</td>
<td>0.61 ± 0.02</td>
<td>2 1.28</td>
</tr>
<tr>
<td>d-Propranolol</td>
<td>3</td>
<td>0.27 ± 0.07</td>
<td>5 1.09 ± 0.10</td>
</tr>
<tr>
<td>l-Propranolol</td>
<td>3</td>
<td>0.35 ± 0.01</td>
<td>4 0.96 ± 0.13</td>
</tr>
<tr>
<td>d,l-Propranolol</td>
<td>4</td>
<td>0.36 ± 0.03</td>
<td>3 1.25 ± 0.02</td>
</tr>
<tr>
<td>Methoxamine</td>
<td>1</td>
<td>0.35</td>
<td>3 1.25 ± 0.02</td>
</tr>
</tbody>
</table>

Table 1: Effects of Media Containing Experimental Agents on Angiotensin I Radioimmunoassay and Angiotensin I Generation from Substrate

Apparent angiotensin I generation refers to the results of the angiotensin I radioimmunoassay after the inclusion of 0.05 ml of medium containing the experimental agent. Substrate studies were performed by adding the medium containing the experimental agent to 0.1 ml of hog renin substrate in the absence of renin during incubation and subsequent assay for angiotensin I generation. All values are means ± SD.

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Effect of the addition of ascorbic acid to incubation medium containing epinephrine (top) and norepinephrine (bottom) on renin release. The length of the bars indicates the mean increase in renin release compared with the control release without the catecholamine; the brackets indicate ±1 SD. The number of experiments is indicated at the base of each bar.

ing stimulation of renin release by epinephrine and norepinephrine. Significant stimulation of renin release was observed with $1.5 \times 10^{-8}$ M epinephrine and norepinephrine in the presence of ascorbic acid ($P < 0.01$). No significant effect on renin release was observed with the same concentrations of epinephrine and norepinephrine in the absence of ascorbic acid. Ascorbic acid alone had no effect on renin release. Mean renin release ± SE was $14.5 ± 0.5$ ng/mg before and $16.7 ± 2.1$ ng/mg during incubation with ascorbic acid-containing medium ($N = 4$).

**EFFECTS OF CATECHOLAMINES ON RENIN RELEASE**

The renin release caused by the addition of catecholamines to the medium containing ascorbic acid during the experimental period was compared with that during the preceding control period. The effects of various concentrations of epinephrine, norepinephrine, and isoproterenol on renin release are shown in Figure 2. Significant dose-related stimulation of renin release was observed with concentrations of each agonist ranging from $1.5 \times 10^{-7}$ to $2.0 \times 10^{-9}$ M. No significant stimulation of renin release was observed with $1.5 \times 10^{-10}$ M epinephrine or norepinephrine or with $2.0 \times 10^{-10}$ M isoproterenol. The comparative dose-response relationships between epinephrine, norepinephrine, and isoproterenol and renin release are shown in Figure 3. There were no significant differences in the response of renin release to the three agonists.

Methoxamine also stimulated renin release at a concentration of $10^{-6}$ M (Table 2).

**EFFECTS OF ADRENERGIC BLOCKING AGENTS ON CATECHOLAMINE-INDUCED STIMULATION OF RENIN RELEASE**

The addition of $d,l$-propranolol or phentolamine had no significant effect on renin release ($17.8 ± 1.7$ ng/mg and $20.0 ± 2.9$ ng/mg, respectively) compared with that during the control observations in media devoid of the antagonists ($16.4 ± 3.6$ ng/mg and $18.2 ± 1.7$ ng/mg, respectively, $N = 4$). The effect of adrenergic blocking agents on catecholamine-induced stimulation of renin release was studied by adding the catecholamines to the media...
which contained the blocking agents and comparing the renin release with that during the preceding control period in medium which contained only the blocking agent. Phenolamine (9 × 10⁻⁴M) had no inhibitory effect on catecholamine-induced stimulation of renin release (Table 2). The effects of d-, d,l-, and l-propranolol (2 × 10⁻⁴M) on catecholamine-induced stimulation are also shown in Table 2.

### Table 2

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine (1.5 × 10⁻⁹M)</td>
<td>14.7 ± 1.1</td>
<td>32.2 ± 4.3*</td>
</tr>
<tr>
<td>Norepinephrine (1.5 × 10⁻⁹M)</td>
<td>10.7 ± 0.6</td>
<td>26.4 ± 4.3†</td>
</tr>
<tr>
<td>Isoproterenol (2 × 10⁻⁹M)</td>
<td>18.7 ± 2.1</td>
<td>39.9 ± 7.3*</td>
</tr>
<tr>
<td>Methoxamine (10⁻⁶M)</td>
<td>7.8 ± 1.7</td>
<td>11.9 ± 2.0*</td>
</tr>
</tbody>
</table>

Observations are expressed as mean renin release (ng/mg kidney) ± SE. All experiments utilized four slices except for those with norepinephrine with d,l-propranolol where eight slices were used. Statistical significance was evaluated by the paired t-test.

* P < 0.01.
† P < 0.02.
‡ P < 0.05.

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2. Catecholamine-induced stimulation of renin release was not affected by d,l-propranolol but was markedly inhibited by both d,l- and l-propranolol. Lower concentrations of d,l-propranolol (2 × 10⁻⁷ and 2 × 10⁻⁸M) did not inhibit isoproterenol-induced stimulation of renin release. Significant stimulation (33.2 ± 4.5 and 45.2 ± 7.0 ng/mg) was observed compared with the control renin release (19.3 ± 1.8 and 16.6 ± 1.4 ng/mg) for these two concentrations. Eight slices were utilized for each study.

The effects of phentolamine in combination with d,l-propranolol on epinephrine- and norepinephrine-induced stimulation of renin release were similar to those observed with propranolol alone (Table 2). Methoxamine-induced stimulation of renin release was blocked by d,l-propranolol but not by phentolamine. The presence of methoxamine inhibited isoproterenol-induced stimulation of renin release as shown in Table 2.

**Discussion**

It has been shown that renal nerve stimulation or infusion of catecholamines stimulates renin release in vivo (4–9). Observations in isolated juxtaglomerular cells (15) have suggested that this stimulation of renin release is a direct effect of catecholamines on these cells rather than an effect secondary to catecholamine-induced changes in hemodynamic or other circulating stimuli. Previous studies utilizing an in vitro system have shown that pharmacologic concentrations of catecholamines can directly stimulate renin release (14, 16, 17).

The present study was designed to further characterize the effects of adrenergic agonists and antagonists on renin release by rat kidney slices in vitro. The variability of absolute renin release among kidney slices can be seen in Figure 2. The utilization of each slice as its own control enabled the detection of significant effects on renin release which might have been masked by differences in basal renin release among slices. Catecholamines are known to be unstable at alkaline pH (19). To prevent degradation of the amines, an antioxidant, ascorbic acid, was added to the medium. As shown in Figures 1–3, the present study demonstrated the effect of catecholamines on renin release at lower concentrations than have previous reports (14–17). These concentrations are comparable to those observed in rat plasma for epinephrine (5 × 10⁻⁴M) and norepinephrine (1 × 10⁻⁴M) in vivo (18). Furthermore, significant dose-related stimulation of renin release by epinephrine, norepinephrine, and isoproterenol in low concentrations was observed, as shown in Figures 2 and 3. These observations confirm that ascorbic acid is important in preventing oxidation of catecholamines and demonstrate that extension of the range of response permits evaluation of the effects of such agents in concentrations similar to those reported in rat plasma (18). The different amines were equipotent in stimulating renin release. This situation is similar to that for a β-type adrenergic receptor described by Furchgott in the rabbit duodenum and stomach (22). This receptor type is different from that found in the pulmonary and cardiovascular systems with respect to differential sensitivity to the three agonists. To further characterize the nature of the adrenergic receptor responsible for renin release in the in vitro kidney slice system, studies with specific adrenergic antagonists were performed.

The β-blocking agents, d,l-propranolol and l-propranolol, inhibited catecholamine-induced stimulation of renin release in vitro. d-Propranolol, which does not have specific β-receptor blocking activity but is as potent an anesthetic agent (23) as is l-propranolol, was ineffective in inhibiting catecholamine-induced stimulation of renin release in vitro. The concentrations of propranolol used were above the dose-response requirements for blocking the effects of 10⁻⁵M agonists as evidenced by the comparable effects of the l- and the racemic formulation. The lack of inhibition of isoproterenol-induced renin release with 2 × 10⁻⁷ and 10⁻⁸M d,l-propranolol suggests that this antagonist is not equipotent with the agonists. The α-receptor blocking agent, phentolamine, failed to suppress renin release stimulated by epinephrine, norepinephrine, or isoproterenol.

Additional experiments were performed with methoxamine, an α-agonist thought to have β antagonistic characteristics (24). Methoxamine at a concentration of 10⁻⁸M induced a significant stimulation of renin release which was abolished by propranolol but not by phentolamine. Methoxamine also inhibited isoproterenol-induced renin release. These β-mediated effects of methoxamine are similar to the effects of the partial agonist dichloroisoproterenol, which is a well-characterized β-agonist with prominent β-receptor stimulant action (25). These observations indicate that methoxamine may be a partial agonist for the β-receptor that mediates renin release. These results also suggest that the effects of adrenergic agonists on renin release in vitro are mediated via a specific β-recep-
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Considerable controversy exists concerning the adrenergic mechanism that mediates renin release in vivo and whether this effect is on the juxtaglomerular cells directly or an indirect effect of alterations in renal hemodynamics or sodium metabolism. Isolated systems, uninfluenced by the latter factors, provide an opportunity to examine direct effects of such agents. Michelakis et al. (15) first demonstrated that norepinephrine can directly stimulate renin release in an in vitro kidney preparation, but they did not characterize the specificity of the receptor further. We have previously shown that catecholamines are capable of stimulating renin release in the rat kidney slice in vitro (14). This observation was confirmed by Nolly et al. (17), who also found that the release of renin induced by norepinephrine and epinephrine is blocked by propranolol and stimulated by phentolamine, phenoxybenzamine, and theophylline. Since these earlier studies used large doses of catecholamines and since no protective agents were used, we suggest that the potentiating effect of $\alpha$-receptor blockers reported by these investigators was due to the uptake blockade induced by the $\alpha$-receptor antagonists (26) rather than to an inhibitory effect of $\alpha$-receptor stimulation on renin release.

Using an isolated rat kidney system perfused at a constant flow rate, Vandongen and co-workers (27) initially reported that the stimulation of renin release by norepinephrine and isoproterenol was blocked by propranolol but not by phenoxybenzamine. In subsequent studies, these investigators were able to demonstrate a stimulatory effect of norepinephrine only in the presence of phenoxybenzamine (28). Moreover, this effect could be abolished by propranolol. Additionally, they demonstrated a partial inhibition of isoproterenol-induced renin release by methoxamine. These observations were interpreted by the authors as indicating a $\beta$-stimulatory and an $\alpha$-inhibitory effect of catecholamines on renin release. In the second study (28), the inability to demonstrate stimulation of renin release by norepinephrine alone could conceivably have been related to rapid oxidation of norepinephrine, since no protecting agents, such as ascorbic acid, were used. An alternative explanation is also possible. It is known that phenoxybenzamine can induce uptake blockade (26); this phenomenon may have permitted a critical level of stimulation of the $\beta$-receptor, which, because of uptake of norepinephrine, was not achieved in the experiments with norepinephrine alone. The present study indicates that methoxamine inhibits isoproterenol-induced renin release by a $\beta$-adrenergic blockade and not by $\alpha$-adrenergic stimulation, which further substantiates the hypothesis that the control of renin release by catecholamines is a pure $\beta$-adrenergic effect.

Johnson et al. (6) have investigated the effects of intra-arterial injections of catecholamines into the denervated, nonfiltering dog kidney. They have found that norepinephrine and epinephrine both stimulate renin release when they are infused at a rate sufficient to reduce renal blood flow to 50% of control. To accomplish this comparable reduction, the investigators utilized different amounts of catecholamines. The norepinephrine infusion rate was 60 $\mu$g/min and that for epinephrine was 40 $\mu$g/min (6). These authors interpreted their data as being consistent with a direct effect of norepinephrine and an indirect effect of epinephrine mediated via vascular factors, because papaverine inhibited only epinephrine-induced stimulation of renin release. Since the present study demonstrated that norepinephrine and epinephrine are equipotent as direct stimuli of renin release, an alternative interpretation of the previous study (6) as a dose-response phenomenon is possible, since less epinephrine was infused than norepinephrine. Thus, the previously cited studies all provide evidence in support of a pure $\beta$-adrenergic receptor for the direct release of renin when differences in experimental design are considered.

Studies of factors influencing renin release in the intact animal and in humans have demonstrated a role for both $\alpha$- and $\beta$-adrenergic stimuli. Such studies further suggest that some of the adrenergic effects, particularly those attributed to stimulation, may be mediated by the hemodynamic effects of such agents. The in vitro system utilized in the present study has been shown to be unresponsive to changes in ionic concentrations of sodium, potassium, or calcium in the incubation medium even though these ions are known to influence renin release in vivo (14). Thus, it has been postulated that such ionic effects require active tubular function and an intact filtration process to influence renin release, since they do not appear to influence renin release directly in the slice system utilized. Moreover, the present results have established a major and direct role for $\beta$-adrenergic stimulation of renin release which appears to be specific and sensitive. This isolated in vitro system has potential usefulness in permitting the precise examination of direct effects of adrenergic stimuli on renin...
release and the dissociation of factors which act in an indirect fashion by alterations in hemodynamic, metabolic, or circulating factors. The nature of this preparation does not preclude the possibility that other substances, such as components of the kallikrein-kinin or prostaglandin systems, may participate directly or indirectly in influencing renin release. Such substances, released from 10–20 mg of renal tissue, would undergo dilution in the incubation medium.

The present study has defined a highly sensitive \( \beta \)-adrenergic receptor within the rat kidney which is capable of stimulating renin release. This receptor is stimulated to an equivalent degree by norepinephrine, epinephrine, and isoproterenol and is totally blocked by propranolol. The study fails to support a role for an \( \alpha \)-adrenergic effect on renin release in this isolated system. The finding of propranolol-inhibited methoxamine stimulation of renin release in this pure \( \beta \)-adrenergic system and the inhibition of isoproterenol-induced renin release by methoxamine suggest that this agent may be a partial \( \beta \)-agonist, having \( \beta \)-agonistic as well as the \( \beta \)-antagonistic properties previously postulated (24). A comparison of the dose-response curves of the present study with the known concentrations of such circulating agents.

Acknowledgment

The authors wish to express their appreciation for the advice of Dr. Louis Lemberger in these studies. We are grateful for the expert assistance of Mrs. Irene Rosevear in preparation of the manuscript.

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Circulation Research. Vol. 37, September 1975
Direct effect of beta-adrenergic stimulation on renin release by the rat kidney slice in vitro.
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*Circ Res.* 1975;37:318-324
doi: 10.1161/01.RES.37.3.318

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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