Published in final edited form as:

Horm Mol Biol Clin Investig. 2010 August ; 2(2): 249–255. doi:10.1515/HMBCI.2010.028.

Adrenaline: communication by electron emission. Effect of concentration and temperature. Product analysis

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Abstract

Background—Based on the recent findings about the ability of sexual hormones to emit electrons (e_{aq}^-) and to act as electron mediator, it was of interest to investigate adrenaline as an important neurotransmitter.

Materials and methods—Highest purity adrenaline (ADR) and chemicals were used for preparation of aqueous solutions (pH ~7.4). The excitation of ADR in singlet state was achieved by irradiation of airfree aqueous solution with monochromatic UV light at $\lambda = 254$ nm. The emitted “solvated electrons” (e_{aq}^-) were scavenged by chloroethanol, where the quantum yield of Cl^- ions, $Q(Cl^-) = Q(e_{aq}^-)$. ADR degradation and formation of photolytic products were followed by HPLC analysis.

Results and conclusion—It was found that $Q(e_{aq}^-)$ values decrease with increasing ADR concentration: for 2.5×10^{-5} mol/L ADR was determined as $Q(e_{aq}^-) = 6 \times 10^{-3}$, whereas for 1×10^{-3} mol/L ADR was found to be 0.9×10^{-3} . This is explained by formation of associates in ground state, which consume a part of emitted e_{aq}^- . As a main photolytic product aminochrome was determined.

Keywords

adrenaline; biological communication; electron consumer; electron emitter; electron mediator

Introduction

Recently, our research group has established for the first time the ability of steroid hormones, such as 17 β -estradiol (17 β E2), progesterone (PRG) (1), as well as testosterone (TES) (2) and the phytohormone genistein (GEN) (3,4), to eject electrons (e_{aq}^-) in polar media from their excited single state. This ability depends on the hormone molecular structure, concentration, temperature and pH of the polar media. The investigated hormones partly consume as well as transfer electrons to other biological systems. Hence, the hormones are also acting as “electron mediators”. This phenomenon allows communication of the hormones with other systems in an organism via the brain and is achieved without forming complexes with receptors (5). It should also be noted that the hormone transients,

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resulting from the electron emission process of the above-mentioned substrates, can lead to metabolite formation. Apart from this, it was found that some of the hormone metabolites can initiate cancer (6,7).

Taking into consideration the above-mentioned premises observed for steroid and phytohormones, it appeared of interest to also investigate adrenaline (ADR) as a representative of the neurotransmitter-hormone group in this respect.

Adrenaline (ADR; epinephrine; (R)-4-[1-hydroxy-2(methylamine)-ethyl]1,2-benzenediol) is an endogenous catecholamine with combined α - and β -agonist activity and is a sympathomimetic hormone and a neurotransmitter, produced by the adrenal medulla. The biological action of ADR in an organism is a rapid preparation of the body for action in emergency situations by boosting the supply of oxygen and glucose to the brain and muscles, as well as simultaneously suppressing other non-emergency processes. It should also be mentioned that ADR is implemented therapeutically for treatment of cardiac dysrhythmics to increase cardiac rate and output as well as for treating anaphylaxis, sepsis, etc. (8).

By contrast, ADR affects some processes in cancer treatment, e.g., an increased uptake of 5-fluorouracil in tumors is observed by simultaneous administration of ADR (9); it influences tumor growth (10,11). Obviously, ADR has a multifunctional activity in the organism. Thereby, it is assumed that the transients resulting by the emission of e_{aq}^- play a determining biological role.

It should also be mentioned that the reaction rate constant of e_{aq}^- with 100% protonated ADR is: $k(e_{aq}^- + ADR-NH_3^+) = 7.5 \times 10^8 \text{ L/mol/s}$ (12,13). Thereby, the acidity constants of ADR are: $pK_{a1}=8.02$, $pK_{a2}=9.46$, $pK_{a3}=10.28$ and $pK_{a4}=13.5$ (14).

The photolysis of ADR using UV light at 254 nm was intensively studied (15–18). As a main degradation product aminochrome and some melanin was found.

Based on the above-mentioned multifunctional biological activity of ADR, the objectives of the present study were mainly focused on the factors governing the electron ejection and the fate of the ADR radicals resulting from this process.

Materials and methods

ADR of highest purity available ($\geq 99\%$ Fluka Aldrich) was dissolved in four-times distilled alkaline water (pH ~ 11) and then the pH was adjusted to 7.4 by means of perchloric acid. At this pH the amino group is protonated. Because the solutions are extremely sensitive to oxygen, even at room temperature, they were freshly prepared each time directly before use and immediately saturated with argon for 30 min prior to irradiation. The UV source was a low-pressure Hg lamp (Osram, HNS 12W) with an incorporated Vycor filter for elimination of the UV-C line at 185 nm. ADR solutions were irradiated with monochromatic UV light of 254 nm (4.85 eV/hv) in a 4π -irradiation apparatus, shown in Figure 1 (19). The ADR solution (200 mL) in the irradiation apparatus is surrounded by water provided by a thermostat keeping a desired constant temperature with a maximum fluctuation of $\pm 1^\circ\text{C}$.

The ejected electrons (e_{aq}^-) were scavenged by $1 \times 10^{-2} \text{ mol/L}$ chloroethanol, resulting in Cl^- ions (see Eq. [1]), which were determined spectrophotometrically as a mercury(II) complex at 460 nm (20). Hence, the obtained $Q(\text{Cl}^-) = Q(e_{aq}^-)$.

The UV intensity at 254 nm was determined using a chloroacetic acid actinometer, where at 30°C the $Q(\text{Cl}^-) = 0.32$ and at 37°C it is 0.41 (22). However, the absorption of the used electron scavenger ($\text{ClC}_2\text{H}_5\text{OH}$) at 254 nm was negligible. The resulting Cl^- yield was taken into account.

Analysis of UV-irradiated 10^{-3} mol/L ADR solution was also performed using the HPLC method. Equipment and procedure used: Hewlett Packard, series 1100 with DAD setup, series 1050; Hypersil ODS column (4.0×125 mm, PN 799 260D-564, batch Lot 4814), mobile phase: 5% acetonitrile, 50% methanol, 45% water, adjusted to pH=2.80 by phosphoric acid; injected sample: 15 µL, flow rate: 1 mL/min.

To obtain a deeper insight into the electron ejection process from excited ADR molecules in airfree, aqueous solution (pH≈7.4), several series of experiments at different temperatures were performed. Firstly, the effect of substrate concentration on the electron yield, $Q(e_{\text{aq}}^-)$, was studied using 2.5×10^{-5} up to 1×10^{-3} mol/L ADR. By application of less than 10^{-5} mol/L ADR, the UV absorption at 254 nm is rather low and the results were not reproducible.

Results and discussion

The determined e_{aq}^- (mol/L) for each of the mentioned ADR concentrations are presented as a function of absorbed UV quanta (I_{abs} , in hv/L) in Figure 2. They represent mean values of several measurements, whereby a standard deviation of approximately 8–10% was observed.

In general, the course of each individual curve showing the yield of e_{aq}^- (mol/L) is linear in respect to the absorbed UV quanta at the beginning, but above a UV dose of approximately 1×10^{21} hv/L, there is a tendency towards a saturation value. This indicates that at lower UV dose the solvated electrons (e_{aq}^-) are generated directly from the substrate. However, the resulting ADR transients lead to the formation of products, which absorb UV light and are likewise able to emit electrons. At a UV dose of approximately 15×10^{21} hv/L a saturation level of e_{aq}^- formation is reached, after which a decrease of the e_{aq}^- yield appears (not given in Figure 2), indicating a rather strong photodegradation of ADR and its products.

The “initial” quantum yields of e_{aq}^- , $Q(e_{\text{aq}}^-)$, were calculated from the tangent of each curve (Figures 2 and 3) and are given as inserts. With increasing ADR concentration the $Q(e_{\text{aq}}^-)$ values are decreasing, as already observed with the other hormones mentioned above (1,2,4). Hence, it is clear, that ADR likewise forms “associates” in the used concentration ranges. In addition to this there are nearly simultaneously competing processes for deactivation of the electronically excited molecules (e.g., fluorescence, phosphorescence, radiationless deactivation, reaction of e_{aq}^- with ADR) proceeding, which consume a part of the excited substrate molecules. The fate of the excited ADR molecules is not studied in this respect in the present study. However, these processes, as well as the relation between them, have been previously investigated by our research group on a number of compounds. The dependence of e_{aq}^- yield upon the various photophysical factors, such as fluorescence, inter-system crossing, etc. (23).

In addition, photophysical processes and e_{aq}^- yields depend on temperature, pH and substrate concentration as previously observed in the case of 17βE2 and PRG (1) as well as

TES (2) and GEN (4). Experiments in this respect were carried out at 30°C, 37°C and 45°C with ADR and are shown in Figure 3.

The curves presenting the yield of e_{aq}^- (mol/L) in dependence of the absorbed quanta (I_{abs} , hv/L) are similar to those in Figure 2. Hence, it can be assumed that the same processes for deactivation of the excited ADR molecules are taking place at the studied temperatures.

It should be finally mentioned that in all experiments the pH of the ADR solutions strongly decreased from 7.4 to approximately 4, depending on the absorbed UV dose.

In accordance with previous investigations of phenolic compounds (23), more electrons are ejected from the π -electron structure of the ring following an intramolecular electron transfer from the OH group (positions 2 and 3) to the ring. Consequently, phenoxyl type of ADR transients are resulting (reaction [2a]), which can lead subsequently to product formation. Additionally, somewhat lower yields of e_{aq}^- can also be emitted from the -OH and amino groups in the ADR side chain. In all cases, the emission of e_{aq}^- is accompanied by an increasing H^+ yield:

With increasing absorbed UV dose the yields of e_{aq}^- and H_{aq}^+ increase at the beginning correspondingly; however, the curves are tending to achieve a saturation value because of substrate photolysis (Figures 2 and 3).

Earlier investigations on ADR photolysis ($\lambda=254$ nm) reported the formation of aminochrome as a main product (16,24). It was now of interest to determine the products from ADR transients, resulting from the electron emission process. Some HPLC analysis of unirradiated and samples irradiated with various absorbed UV doses were performed. In Figure 4, a few chromatograms are shown for illustration of the subject matter.

The absorption spectra of the individual peaks were measured. Based on the characteristic absorption maxima of aminochrome of ADR ($\lambda=301$ and 287 nm) (24), peak 2 of the HPLC chromatogram B, Figure 4 was identified as ADR aminochrome. With increasing absorbed UV dose (chromatogram C, Figure 4) a new peak 3, very close to peak 1, was registered. It was attributed to a mixture of dimers resulting from the mesomere structure given below. Also, a small peak 4 was clearly observed in the same chromatogram.

Considering reaction [2a] as a main source of the e_{aq}^- formation, it should be mentioned that the resulting phenoxyl-radical type of the ring exists in several mesomere structures [Eq. [3] (25)]

Each of these structures can lead to the formation of a final product. The formation of the observed ADR aminochrome (AC) (Figure 4) can be explained by cyclization of the mesomere structure (d) as shown in Eq. [4]:

Reaction [4] also demonstrates that N atoms act as an additional source of electron ejection as previously shown for aniline (23).

The observed peak 3, Figure 4, is attributed to a mixture of dimers resulting from the mesomere structures (Eq. [3]).

At prolonged irradiation in the presence of air aldehydes and carboxylic acids can also be formed from structures (a) to (d), similarly shown for phenol (25).

Based on a number of HPLC analyses of ADR samples treated with various absorbed UV doses, the degradation of ADR and the corresponding formation of aminochrome were determined. The obtained data are shown in Figure 5, where curve (A) represents the photodecomposition of ADR and curve (B) the formation of ADR aminochrome, both as a function of absorbed UV dose. From the slope of curves (A) and (B), the corresponding quantum yields (Q) have been calculated and are presented as an insert in Figure 5. More than 50% of the decomposed ADR is converted into aminochrome.

For clarity, the correlation of determined initial $Q(e_{aq}^-)$ and used ADR concentrations, individual data are compiled in Table 1.

Comparing the obtained $Q(e_{aq}^-)=0.9\times 10^{-3}$ for 10^{-3} mol/L ADR with those of ADR degradation, $Q(-\text{ADR})=5.45\times 10^{-3}$ and the formation of ADR aminochrome, $Q(\text{AC})=2.8\times 10^{-3}$ (see curves A and B in Figure 5), it can be concluded that the cyclization process (reaction [4]) successfully competes with “self-consumption”, a part of the ejected e_{aq}^- , $k(\text{ADR}+e_{aq}^-)=2.5\times 10^8$ L/mol/s (12) and the electron transfer process to other biological systems. In other words, the initial $Q(e_{aq}^-)$ yield of ejected electrons is in reality much higher than that determined by using chloroethanol as an electron scavenger. Hence,

the yield of emitted e_{aq}^- involved in the communication of ADR with other biological systems via the brain is made possible by the main part of the total emitted electrons (5).

Conclusion

The highlights of the present study can be summarized as follows:

- ADR molecules in airfree, aqueous solution (pH ~7.4) can eject electrons (formation of e_{aq}^-) from their excited single state (monochromatic UV light, $\lambda=254$ nm). Simultaneously, the pH of the media is decreased by formation of H^+ ions (reaction [2]).
- The initial quantum yield of affected electrons, $Q(e_{aq}^-)$, depends on substrate concentration and temperature.
- At higher concentrations, $>10^{-5}$ mol/L, ADR forms “associates” (unstable complexes), where the substrate molecules in ground state partly react with the generated e_{aq}^- ($k=2.5 \times 10^8$ L/mol/s) and reduce the $Q(e_{aq}^-)$ value. In addition, the major part of e_{aq}^- can be transmitted via brain centers to other biological systems. Hence, ADR is acting as an “electron mediator”, which enables communication with other biological systems in organisms.
- The predominant part (>50%) of the ADR transients resulting from the electron emission process undergo cyclization, forming aminochrome in addition to dimers and small yields of other unidentified final products.
- Probable reaction mechanisms are given to demonstrate the rather complicated biological processes.

Acknowledgments

This study was partly performed in the framework of the research project “Free Radicals Action on Sexual Hormones in Respect to Cancer” (contract No. P21138-B11), subsidized by FWF (Austrian Science Fund), which is truly acknowledged. N.G. would like to thank Greiner Holding GmbH, KG & AG., Kremsmünster, Austria, for providing radiation sterilized laboratory utensils. We would also like to thank Mrs Haley Ramsey for her efforts for English improvement of the manuscript.

References

1. Getoff N, Hartmann J, Huber JC, Quint RM. Photo-induced electron emission from 17β -estradiol and progesterone and possible biological consequences. *J Photochem Photobiol B Biol* 2008;92:38–41.
2. Getoff N, Schittl H, Hartmann J, Quint RM. Electron emission from photo-excited testosterone in water-ethanol solution. *J Photochem Photobiol B Biol* 2009;94:182–97.
3. Getoff N, Schittl H, Quint RM. Electron emission from genistein in relationship to its anticancer properties [abstract]. Abstracts of the 8th International Conference on Anticancer Research, Kos, 17–22 Oct 2008. *Anticancer Res* 2008;28:3478.
4. Getoff N, Schittl H, Quint RM. Electron emission of phytohormone genistein. Pathway for communication and biological consequences. *J Curr Bioact Compd* 2009;5:215–8.
5. Getoff N. UV-radiation-induced electron emission by hormones. Hypothesis for specific communication mechanisms. *Radiat Phys Chem* 2009;78:945–50.
6. Seeger H, Deutinger FU, Wallwiener D, Mueck AO. Breast cancer risk during HRT: influence of estradiol metabolites on breast cancer and endothelial cell proliferation. *Maturitas* 2004;49:235–40. [PubMed: 15488352]
7. Kraemer EA, Seeger H, Kraemer B, Wallwiener D, Mueck AO. The effect of progesterone, testosterone and synthetic progestogens on growth factor- and estradiol-treated human cancerous and benign breast cells. *Eur J Obstet Gynecol Reprod Biol* 2006;129:77–83. [PubMed: 16460873]

8. Online Medical Dictionary: Epinephrine.
9. Vagianos C, Puntis M, Jeppson B, Steen S, Zoncas E, Bengmard S. Increased uptake of 5-FU in experimental liver tumors by simultaneous infusion of norepinephrine. *Eur J Cancer Clin Oncol* 1987;23:1323–7. [PubMed: 3678326]
10. Lüthy JA, Bruzzzone A, Pinero CP, Castello LF, Chiesu JJ, Vazquez SM, Sauppa MG. Adrenoceptors: non conventional target for breast cancer? *Curr Med Chem* 2009;16:1850–62. [PubMed: 19442150]
11. Yao H, Duan Z, Wang M, Awonuga AD, Rappolee D, Xie Y. Adrenaline induces chemoresistance in H7-29 colon adenocarcinoma cells. *Cancer Genet Cytogenet* 2009;190:81–7. [PubMed: 19380024]
12. Gohn M, Getoff N. Pulse radiolysis of adrenaline in acid aqueous solutions. *Int J Radiat Phys Chem* 1976;8:533–8.
13. Gohn M, Getoff N, Bjerbakke E. Radiation chemistry studies on chemotherapeutic agent. I. Pulse radiolysis of adrenaline in aqueous solutions. *J Chem Soc Faraday Trans II* 1977;73:406–14.
14. Corona-Avendano S, Alareon-Ageles G, Rojas-Hernandes A, Romero-Romo MA, Ramirez-Silva MT. Study of stability of adrenaline and on the determination of acidity constants. *Spectrochim Acta A* 2005;61:305–11.
15. Gerke DC, Crabb GA, Frewin DB. The effect of irradiation and heat on the content of adrenaline in commercially manufactured local anesthetic solution – a pilot study. *Aust Dent J* 1978;23:311–3. [PubMed: 282834]
16. De Mol NJ, Beijersbergeg van Henegouwen GMJ, Gerritsma KW. Photochemical decomposition of catecholamines. II. The extent of aminochrome formation from adrenaline, isoprenaline and noradrenaline induced by ultraviolet light. *Photochem Photobiol* 1979;29:479–82.
17. Thoma K, Struve M. Studies on the photo- and thermostability of adrenaline solutions. Part 1. Stability of adrenaline solutions. *Pharmaceutica Acta Heletiae* 1986;61:2–9.
18. Chafetz L, Chow LH. Photochemical hydroxylation of phenylephrine to epinephrine (adrenaline). *J Pharm Biomed Anal* 1988;6:511–4. [PubMed: 16867397]
19. Getoff N. Primary products of water photolysis at 1849 Å (in German). *Monatsh Chem* 1968;99:136–47.
20. Florence TM, Ferra YJ. Spectrophotometric determination of chlorine at the parts-per-billion level by mercury(II)thiocyanate method. *Anal Chem Acta* 1971;54:373–7.
21. Buxton GV, Greenstock CL, Helman WP, Ross AB. Critical review of rate constants for reaction of hydrated electrons, hydrogen atoms and hydroxyl radicals in aqueous solution. *J Phys Chem Ref Data* 1988;17:513–86.
22. Neumann-Spallart M, Getoff N. Photolysis of monochloric acetic acid at 253.7 nm in aqueous solution (in German). *Monatsh Chem* 1975;106:1359–67.
23. Getoff N. A review of the relationship between $Q(e_{aq}^-)$ and $Q(F)$ of excited compounds in aqueous solution. *Radiat Phys Chem* 1989;34:711–9.
24. Heacock RA, Mattok GL. The chemistry of the aminochromes. Part V. Rearrangements in the presence of sodium hydroxide and zinc acetate. *Can J Chem* 1963;14:139–46.
25. Getoff N. Radiation-induced decomposition of water pollutants. State of the art. *Radiat Phys Chem* 1996;47:581–93.

Figure 1. Apparatus for UV irradiation of solutions using a low-pressure Hg lamp (OSRAM, HNS 12) with an incorporated Vycor filter for elimination of 185 nm line (19).

Figure 2.

Yield of solvated electrons (e_{aq}^- , mol/L) at various ADR concentrations in airfree solution (pH ~7.4, 37°C) in dependence of absorbed UV quanta (I_{abs} , hv/L; $\lambda=254$ nm). (Inserts) above: structural formula of ADR; below: initial quantum yields of e_{aq}^- , calculated from the starting linear part of the curves A–D.

Figure 3.

Electron emission (e_{aq}^- , mol/L) of 5×10^{-5} mol/L ADR in airfree aqueous solution (pH=7.4) as a function of absorbed quanta (I_{abs} in hv/L) at different temperatures. Insert: initial quantum yields, $Q(e_{aq}^-)$, calculated from the linear part of the particular curve.

Figure 4.

HPLC chromatograms. (A) Unirradiated sample peak 1: 10^{-3} mol/L ADR; peak 2: aminochrome, formed by oxidation of ADR during the preparation of the solution. (B) Irradiated airfree sample; peak 1: ADR remainder, peak 2; aminochrome; (C) airfree sample irradiated at higher UV dose, peak 1: ADR; peak 2: aminochrome; peak 3: mixture of ADR dimers; peak 4: unknown photolytic product.

Figure 5.

(A) Photoinduced degradation of 10^{-3} mol/L ADR in airfree aqueous solution (pH ~ 7.4) by monochromatic UV light ($\lambda=254$ nm) and (B) formation of ADR aminochrome (AC) both as a function of absorbed quanta ($h\nu/L$). The corresponding initial quantum yields (Q) are given as an insert.

Table 1Quantum yield, $Q(e_{aq}^-)$ emitted at different starting ADR concentrations at 37°C

ADR, mol/L	$Q(e_{aq}^-)$, at 37°C
2.5×10^{-5}	6.0×10^{-3}
5.0×10^{-5}	4.2×10^{-3}
1.0×10^{-4}	2.7×10^{-3}
2.5×10^{-4}	1.4×10^{-3}
1.0×10^{-3}	0.9×10^{-3a}

^aNot given in Figure 2.