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Structure–activity relationship of coordinated catecholamine in the $[Ru^{III}(NH_3)_4(catecholamine)]^+$ complex

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Abstract

The redox chemistry and pharmacological studies of the novel blue ruthenium(III)–catecholamine complexes were investigated in aqueous medium and compared to the free catecholamines. The $[Ru^{III}(NH_3)_4(catecholamine)]^+$ can be oxidized or reduced reversibly in one electron redox couples in aqueous solution. This is in contrast to the free catecholamines, which has a complicated electrochemical behavior due to coupled protonation process. The introduction of the ruthenium group reduces the intrinsic efficacy of the studied catecholamines. The $[Ru^{III}(NH_3)_4(catecholamine)]^+$ complex aqueous medium is more stable than the free catecholamines ligand in the same conditions.

Keywords: Catecholamines; Ruthenium(III) complexes; Dioxolene ligands; Quinone

1. Introduction

There has been a significant interest in the investigation of the electrochemistry of catecholamines due to the important neurological and hormonal functions that these compounds perform in mammals. The catecholamines (Fig. 1) are neurotransmitters that regulate the cellular activity via interaction with adrenergic receptors (Hawley et al., 1967; Brun and Rosset, 1974; Wightman et al., 1988).

The molecular requirement for catecholamines binding to the adrenergic receptors occurs due to several interactions. Among them, we can point out the electrostatic interaction between the receptor and the amino group, β-hydroxyl bonds, and Van der Waals attractive interactions (Barger and Dale, 1910; Cavalli et al., 1996; Haw and Perez, 1996; Cotecchia et al., 2000). As free ligands, the catecholamine species undergo a primary electrochemical oxidation process, resulting in quinone products that are susceptible to pH dependency and secondary chemical reactions. One of these secondary products is neuromelanine (Fig. 2), which is involved in

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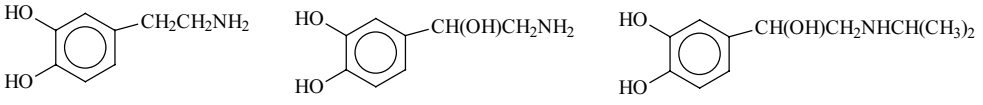


Fig. 1. Schematic catecholamines structures.

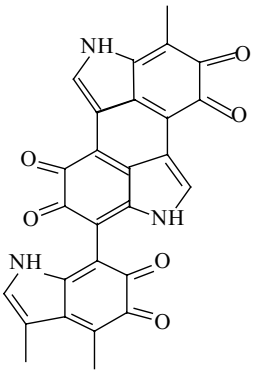


Fig. 2. Neuromelanine structure.

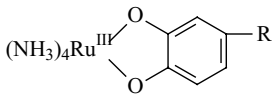
the development of Parkinson's disease (Drukarch and Van Muiswinkel, 2000; Linert and Jameson, 2000).

Although the hormonal actions and functions of the catecholamines have been extensively studied, detailed information regarding their interactions with metal producing complexes, the effect of this coordination on the pharmacological activity, and their electrochemical behavior after this coordination are still uncertain.

In order to understand the electrochemical and pharmacological behavior of catecholamines (a catechol type ligand) when it is coordinated to a metal, we have synthesized $[Ru^{III}(NH_3)_4(catecholamine)]^+$ (Fig. 3), where catecholamines are noradrenaline, dopamine and isoproterenol, in a way that only the catechol form is generated in the presence of the amineruthenium(III) species.

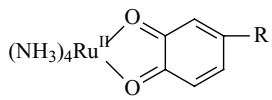
These catecholamine complexes may exist in a variety of metal oxidation states and three oxidation states of the dioxolene ligand(s) (quinone, semiquinone and catechol) linked together within a redox series (Silva et al., 1995, 2000). Since the ruthenium ion can bind to catecholamines via the catechol hydroxyl groups at positions 3 and 4, a change can be expected to occur in the biological effects of catecholamines.

The aim of the present study is to understand the effect induced by the coordination of catecholamine to a transition metal. To evaluate this effect we performed pharmacological assays in the rat anococcygeus muscle, a tissue that is richly endowed by catecholamine receptors mediating contractile responses. Assays are performed with catecholamines in both free and coordinated forms.



$[Ru^{III}(NH_3)_4(catecholamine)]^+$

(a)



$[Ru^{II}(NH_3)_4(catecholamine\text{-}quinone)]^{2+}$

(b)

-R	ligand
$-COO^-$	3,4-diolatobenzoato
$-CH_2CH_2NH_2$	dopamine
$-CH(OH)CH_2NH_2$	noradrenaline
$-CH(OH)CH_2NHCH(CH_3)_2$	isoproterenol

Fig. 3. Schematic ruthenium complexes structures: (a) $[Ru^{III}(NH_3)_4(catecholamine)]^+$ and (b) $[Ru^{II}(NH_3)_4(catecholamine\text{-}quinone)]^{2+}$.

2. Experimental

2.1. Chemicals

The complex $[Ru^{III}(NH_3)_5Cl]Cl_2$ was prepared using a published procedure (Allen and Senoff, 1967). The species noradrenaline, isoproterenol and dopamine were obtained from Aldrich and used as supplied.

2.2. Preparation of complexes

The $[Ru^{III}(NH_3)_4(catecholamine)](PF_6)$ complex, where catecholamine is noradrenaline, dopamine and isoproterenol, was synthesized as previously described (Lima et al., 2003). The purity of the complexes was checked by HPLC analysis, characterized by UV-Vis and infrared spectroscopy, and then compared to the published results (Lima et al., 2003). UV-Vis spectra: λ (nm) ($\log \varepsilon$) $[Ru^{III}(NH_3)_4(dopamine)]^+$: 280 (3.50), 319 (2.83), 429 (2.60), 680 (3.02) and FTIR $\nu_{(C-O)}$ 1282 cm^{-1}; $[Ru^{III}(NH_3)_4(noradrenaline)]^+$: 290 (3.55), 360 (3.42), 672 (3.30) and FTIR $\nu_{(C-O)}$ 1284 cm^{-1}; $[Ru^{III}(NH_3)_4(isoproterenol)]^+$: 289 (3.63), 330 (3.32), 423 (2.80), 666 (3.34) and FTIR $\nu_{(C-O)}$ 1287 cm^{-1}.

The $[Ru^{II}(NH_3)_4(quinone\text{-}COO)](PF_6)$ (Fig. 3) complex was synthesized by the controlled potential eletrolysis oxidizing $[Ru^{III}(NH_3)_4(cat\text{-}COO)]$ in 2.0×10^{-1} M HPF_6 aqueous solution where cat-COO is 3,4-diolatobenzoato. The $[Ru^{II}(NH_3)_4(quinone\text{-}COO)](PF_6)$ complex was separated by filtration after standing for 5 h in a freezer and washed with ethanol. Yield 50%. Calc.: For $[Ru^{II}(NH_3)_4(quinone\text{-}COO)](PF_6)$: C, 17.39; H, 3.51; N, 11.59. Found: C, 17.39; H, 3.87; N, 11.15.

2.3. Physical measurement

The electronic spectra were recorded on a UV-Vis-NIR Hitachi model U-3501 spectrophotometer. Cyclic and differential pulse voltammetry measurements were carried out with an AUTOLAB® model PGSTAT 30 potentiostat/galvanostat, by using a conventional electrochemical cell containing a glassy carbon working electrode, an Ag/AgCl reference electrode, and a platinum wire auxiliary electrode. NaTFA/HTFA (where TFA^- is trifluoroacetate), NaAc/HAc (where Ac^- is acetate) and NH_4Cl/NH_4OH were employed as buffer solution and in 0.1 M KCl in aqueous solution as a supporting electrolyte. The spectroelectrochemistry measurements were carried out using an AUTOLAB® model PGSTAT 30 potentiostat/galvanostat in parallel with a UV-Vis-NIR Hitachi model U-3501 spectrophotometer. A three-electrode system was designed for a rectangular quartz cell of 0.500 mm internal optical path length. A gold minigrid was used as a transparent working electrode, in the presence of a Ag/AgCl reference electrode and a platinum auxiliary electrode. The cell was located directly in the spectrophotometer, and the absorption change was monitored during the electrolysis. All the measurements were carried out at 298 K. The pH measurements were carried out with a digital pH meter DIGIMED DM-20.

2.4. Pharmacological assays

Adult male Wistar rats, weighing 200–250 g, were decapitated and the anococcygeus muscle removed (Gillespie, 1972). Tissues were placed in 5 ml of physiological salt solution (PSS) containing (mM): NaCl 118, KCl 4.7, $NaHCO_3$ 25, $MgSO_4$ 0.45, KH_2PO_4 1.03, $CaCl_2$ 2.5, D-(+)-glucose 11.1. The PSS was gassed with 5% CO_2 and 95% O_2 and maintained at 37 °C, pH 7.4, and periodically checked. Isotonic transducers were used to measure the changes in the isotonic tension of the tissues, which were displayed on a Harvard Universal Student Oscillograph (MA, USA) at a resting tension of 1 g. Isolated muscles were allowed to equilibrate for 45 min before making experimental observations. The organ bath PSS was repeatedly replaced with fresh PSS every 15 min. After the equilibration period, tissues were stimulated with KCl (90 mM) to check their responsiveness. Cumulative concentration-effect curves for free catecholamines and for the ruthenium catecholamine complexes (0.10 nM–1 mM) were obtained before and after their metal coordination, as well as after the incubation of tissues with coordinated compounds for 30 min.

2.4.1. Data analysis

Contractions were recorded as changes in the displacement (mm) from baseline and expressed as percentage of the tonus induced by KCl (90 mM).

Agonist concentration–response curves were fitted using a non-linear interactive fitting programme (GraphPad Prism 2.00, Graph Pad Software Incorporated). Agonist potencies and maximum response were expressed as pD_2 (logarithm of the molar concentration of agonist producing 50% of the maximum response) and E_{max} (maximum effect elicited by the agonist), respectively. Statistical significance was determined by using the one-way analysis of variance (ANOVA) and Student's *t*-test. In all cases, probability levels of less than 0.05 ($P < 0.05$) were taken to indicate statistical significance.

Table 1
Spectroscopic and electrochemical data for $[Ru(NH_3)_4(dioxolene)]^{n+}$ specie

Dioxolene	$E_{1/2(A)}$ (V)[a]	$E_{1/2(B)}$ (V)[a]	$E_{1/2(C)}$ (V)[a]	λ (nm)
cat-COO	0.32	–	−0.69	210; 265; 298; 660
nora[b]	0.24	−0.35	−0.70	290; 360; 672
dopa[c]	0.20	−0.35	−0.80	280; 360; 680
q-COO	–	–	–	286; 460; 520

A: quinone/catechol process; B: amine/imine process; C: $Ru^{(III)/II}$ process.
[a] Potential vs. Ag/AgCl, obtained at pH 7.0.
[b] nora: noradrenaline.
[c] dopa: dopamine.

3. Results and discussion

The transition metal chemistry in the biological activity of catecholamines helps explain the pharmacological mechanisms by which the effects are produced. The catecholamine ligand is bonded to ruthenium(III) producing $[Ru^{III}(NH_3)_4(catecholamine)]^+$ with a stable complex in aqueous solution at least for one week. As the biological activity of the complex depends mainly on the structure in this study, some pharmacological results are evaluated and compared to free catecholamines. The electrochemical behavior of the complexes was also studied once it was known that the ligand could have three different oxidation states, which can help explain the activity of the species during the pharmacological assays.

The $[Ru^{III}(NH_3)_4(catecholamine)]^+$ complexes where catecholamines is dopamine, isoproterenol and noradrenaline, were prepared by treating $[Ru^{III}Cl(NH_3)_5]Cl_2$ with the corresponding catecholamine in the presence of base under argon atmosphere in a similar process previously described for dioxolene complexes (Silva et al., 1995, 2000; Lima et al., 2003).

The cyclic voltammograms of the dioxolene ligand complexes in aqueous solution show multiple couples, which result from the redox processes centered

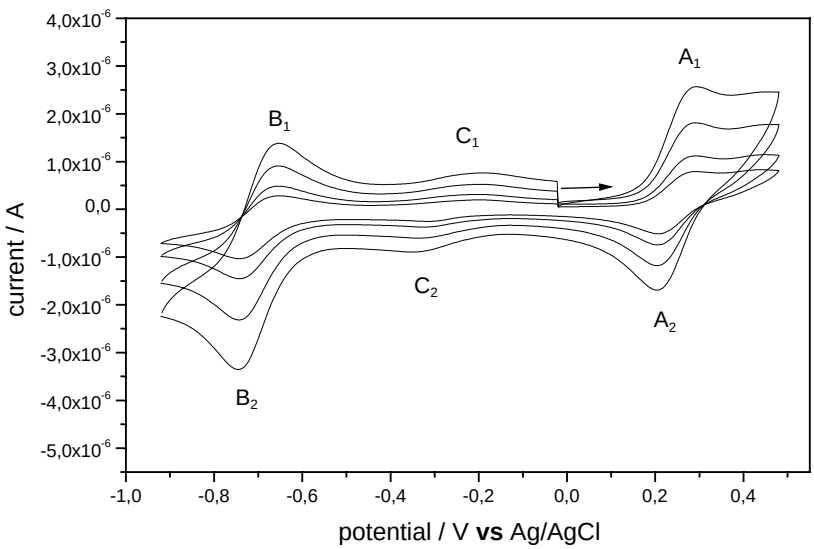


Fig. 4. Cyclic voltammograms of $[Ru^{III}(NH_3)_4(noradrenaline)]^+$ 1.0×10^{-3} M; aqueous KCl 0.20 M at pH 5.80. Scan rates 20, 50, 100, 200, 300 mV s^{-1}.

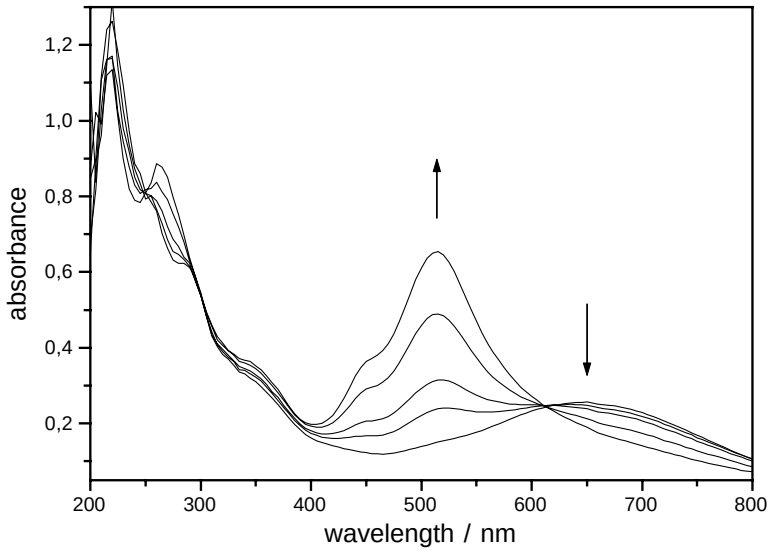


Fig. 5. Spectroelectrochemistry of $[Ru^{III}(NH_3)_4(noradrenaline)]^+$ 1.0×10^{-4} M aqueous KCl 0.20 M at pH 7.00; applied potential +0.50 V vs. Ag/AgCl.

at the metal and the catecholamine ligand. The cyclic voltammetry data for $[Ru^{III}(NH_3)_4(catecholamine)]^+$, are listed in Table 1. A representative cyclic voltammogram is showed for $[Ru^{III}(NH_3)_4(noradrenaline)]^+$ in Fig. 4. The peak A_1 and A_2, in Fig. 4, were attributed to the process centered on the catechol ligand associated with *o*-quinone formation. The redox process centered on the metal appears in negative region of the cyclic voltamogramm and could be attributed by the peaks B_1 and B_2. The peaks C_1 and C_2 were attributed to the process centered on the amine group in catechol site. The Nicholson–Shain criteria (I_{pa} vs. scan rate; $I_{pa}/I_{pc} = 1$; and peak–peak separation near 60 mV) (Nicholson and Shain, 1965) were used to diagnose the couples as reversible, one electron processes in all studied complexes.

The assignment of the redox process was made based on the spectroelectrochemistry experiment and by comparison to $[Ru^{III}(NH_3)_4(cat\text{-}COO)]\cdot 2H_2O$ (Silva et al., 1995). The electronic absorption spectrum of $[Ru^{III}(NH_3)_4(catecholamine)]^+$ exhibited a strong absorption band in the 660 nm region. Indeed, the electrochemical oxidation of $[Ru^{III}(NH_3)_4(noradrenaline)]^+$, for example, at 0.50 V versus Ag/AgCl brings about the appearance of the 520 nm band at the expense of the 660 nm band of $[Ru^{III}(NH_3)_4(noradrenaline)]^+$ as observed in Fig. 5. The electronic spectrum of oxidized $[Ru^{III}(NH_3)_4(noradrenaline)]^+$ is very similar to the electronic spectrum of synthesized $[Ru^{II}(NH_3)_4(quinone\text{-}COO)](PF_6)$ (Table 1). Based on the electronic spectrum, the configuration of the oxidized specie is reasonably expressed by $[Ru^{II}(NH_3)_4(noradrenaline\text{-}quinone)]^{2+}$. The full electrochemical reduction of $[Ru^{III}(NH_3)_4(noradrenaline)]^+$ observed at −0.60 V versus Ag/AgCl resulted in the disappearance of the 660 nm band (Fig. 6). The oxidation of the resultant solution at 0.00 V versus Ag/AgCl regenerated the electronic spectrum of $[Ru^{III}(NH_3)_4(noradrenaline)]^+$, indicating that the reduced complex is a chemically stable specie at pH 7.0 and probably should be described as $[Ru^{II}(NH_3)_4(noradrenaline)]$.

A partial reduction of $[Ru^{III}(NH_3)_4(catecholamine)]^+$ can be observed in the region of −0.40 to 0.00 V versus Ag/AgCl (Table 1). The peak "C" on the cyclic voltammogram of $[Ru^{III}(NH_3)_4(catecholamine)]^+$ (Fig. 4) can be attributed to the process centered in the amine group of the catecholamine ligand. The assignment was made based on a similar process observed for free catecholamines (Hawley et al., 1967; Brun and Rosset, 1974) and also because it was not observed in the cyclic voltammogram of $[Ru^{III}(NH_3)_4(cat\text{-}COO)]\cdot 2H_2O$ (Silva et al., 1995).

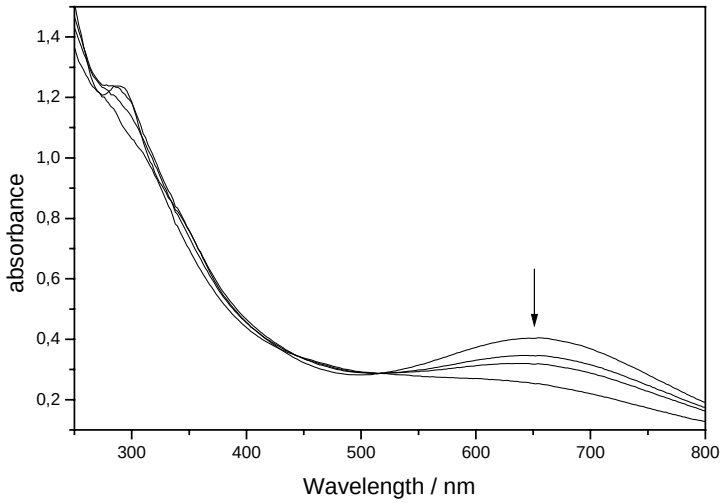
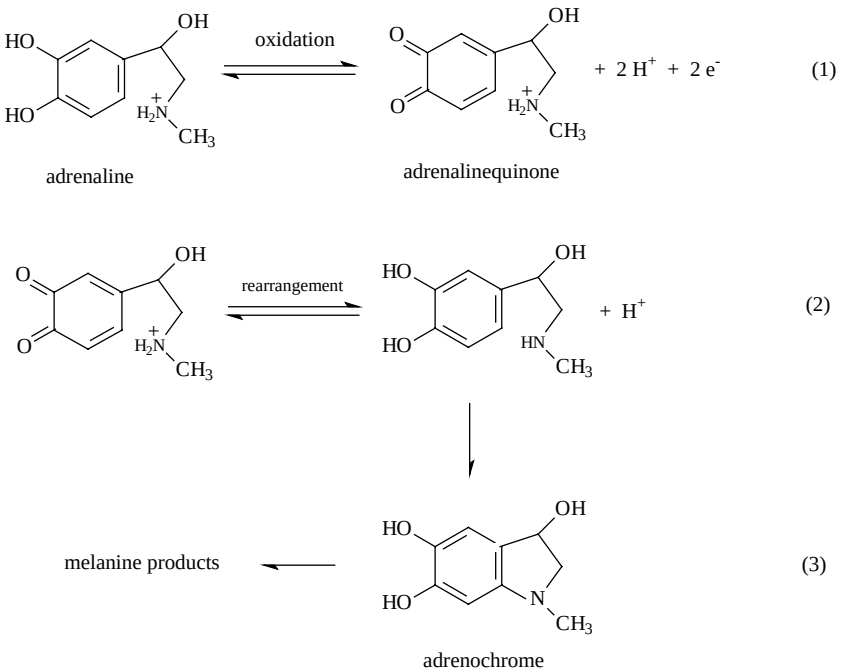


Fig. 6. Spectroelectrochemistry of $[Ru^{III}(NH_3)_4(noradrenaline)]^+$ 1.0×10^{-4} M aqueous KCl 0.20 M at pH 7.0; applied potential −0.90 V vs. Ag/AgCl.

In addition, the spectroelectrochemistry performed in the −0.40 to 0.00 V versus Ag/AgCl region for $[Ru^{III}(NH_3)_4(catecholamine)]^+$ complex does not appreciably change the electronic spectrum of the reduced and oxidized specie. These results are indicative of the oxidation of the amine group on the catecholamine ligand in $[Ru^{III}(NH_3)_4(catecholamine)]^+$ complex.



Scheme 1.

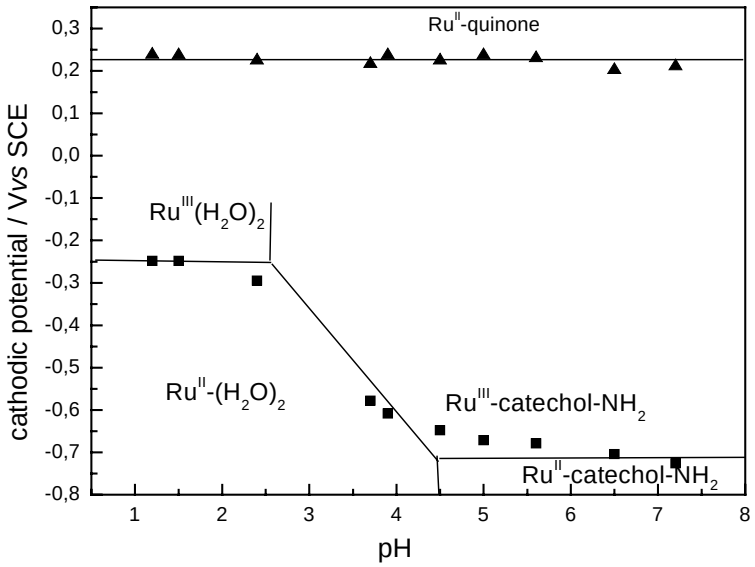


Fig. 7. The pH-potential regions of stability for the various oxidation states of the $[Ru^{III}(NH_3)_4(noradrenaline)]^{+}$. The p$K_a$ value are shown by the vertical line.

The ruthenium(III) ion completely changes the electrochemical behavior of free catecholamines. The oxidation of free ligands allows an irreversible 1,4-intramolecular cyclization (Scheme 1), leading to the formation of aminochromes (Hawley et al., 1967; Remião et al., 2002) that are involved in Parkinson's disease (Drukarch and Van Muiswinkel, 2000; Linert and Jameson, 2000; Martinez-Alvarado et al., 2001; Riobó et al., 2002) and cellular toxicity (Rupp and Dhalla, 1994; Dhalla et al., 1996; Remião et al., 2001). We have no evidence by electronic spectrum that this cyclization of the amino group occurs when the catecholamines ligands are coordinated to the ruthenium(III) as previously observed for some free catecholamines (Senoh and Witkop, 1959). Probably the metal ion stabilizes the catecholamine ligand by removing electron density of the catechol ring, which should impede the cyclization.

The redox couples in the $[Ru^{III}(NH_3)_4(catecholamine)]^{+}$ system are pH dependent (Fig. 7). In acidic medium below pH ca. 4.50 the fully reduced $[Ru^{II}(NH_3)_4(catecholamine)]$ specie dissociates irreversibly to *cis*-$[Ru^{II}(NH_3)_4(H_2O)_2]^{2+}$ in a similar process observed for $[Ru^{III}(NH_3)_4(cat\text{-}COO)]\cdot 2H_2O$ (Silva et al., 1995). By studying the pH dependence of the peak potentials, it was possible to obtain the acid dissociation constant (pK_as) of coordinated catecholamine, which are 4.50, 4.20 and 4.50 for noradrenaline, dopamine and isoproterenol, respectively.

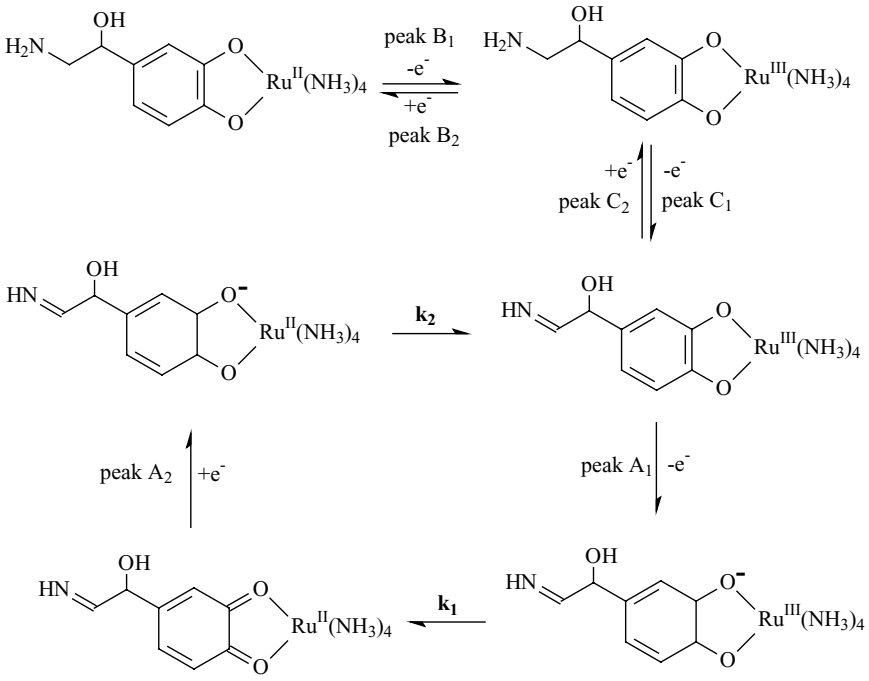
Based on the electrochemical and spectroelectrochemical behavior of the $[Ru^{III}(NH_3)_4(catecholamine)]^{+}$ specie, it is possible to infer the electrochemical mechanism as described in Scheme 2 for $[Ru^{III}(NH_3)_4(noradrenaline)]^{+}$ in neutral pH.

The rate constant k_1 and k_2 (Scheme 2) were assigned as the inter-conversion of $[Ru^{III}(NH_3)_4(noradrenaline\text{-}semiquinone)]^{2+}$ to $[Ru^{II}(NH_3)_4(noradrenaline\text{-}quinone)]^{2+}$ and $[Ru^{II}(NH_3)_4(noradrenaline\text{-}semiquinone)]^{2+}$ to $[Ru^{III}(NH_3)_4(noradrenaline)]^{2+}$, respectively, as described for similar systems (Silva et al., 2000).

3.1. Pharmacological assays

3.1.1. Contractile assays

Noradrenaline induced contractions in the rat anococcygeus muscle with a pD_2 of 5.89(2) and E_{max} of 106.10(1)%. The introduction of the ruthenium group significantly reduced the pD_2 and E_{max} values to 4.24(2) and 76.63(2)%, respectively (Fig. 8A). This reduction of pharmacological activity is probably associated with the impediment of the interaction of catecholamine functional groups with adrenergic receptors once the catechol *meta*- and



Scheme 2.

para-hydroxyl groups are attached to ruthenium(III). The $[Ru^{II}(NH_3)_4$(noradrenaline-quinone)$]^{2+}$ complex was obtained by the electrochemical oxidation of $[Ru^{III}(NH_3)_4$(noradrenaline)$]^{+}$. The $[Ru^{II}(NH_3)_4$(noradrenaline-quinone)$]^{2+}$ complex, where the noradrenaline-quinone is the oxidized form of noradrenaline, was inactive (Fig. 8B). Similar results were found when free catecholamine was oxidized to quinone but in that case the pharmacological inactivity was attributed to the polymerization of catecholamine-quinone (Rupp and Dhalla, 1994; Dhalla et al., 1996; Remião et al., 2001, 2002).

We believe that there is no decomposition of the $[Ru^{II}(NH_3)_4$(noradrenaline-quinone)$]^{2+}$ specie during the pharmacological assays since it can, in situ, be reversibly transformed back into catecholamine. The pharmacological inactivity of $[Ru^{II}(NH_3)_4$(noradrenaline-quinone)$]^{2+}$ in that case could be related to the existent strong back bonding between ruthenium(II) and the quinone group (Silva et al., 2000). The electron density is transferred from Ru(II) to a noradrenaline-quinone ligand, which should reduce the Van der Waals interaction with the adrenergic receptors. The pharmacological results observed for coordinated dopamine and isoproterenol species are quite different from those obtained for coordinated noradrenaline. Contractions in the rat anococcygeus muscle induced by free dopamine produced pD_2 of 5.52(2) and E_{max} of 102.40(2)%, although the $[Ru^{III}(NH_3)_4$(dopamine)$]^{+}$ did not produce any effect (Fig. 8C). The difference observed in the contractile assays between coordinated noradrenaline and coordinated dopamine is probably due to the interaction of hydroxyl substituent of noradrenaline (Fig. 1) with the adrenergic receptor. Moreover, it has been shown that the activity of the free catecholamines is dependent upon the presence of the catechol phenolic hydroxyl groups at positions 3 and 4. When both these groups are substituted or absent, with no other aromatic substitution, the overall potency is reduced. So the introduction of the ruthenium ion may produce a steric impediment, which could make difficult the interaction of the catecholaminergic portion of the complex with the specific receptor, reducing the efficacy and potency of those compounds.

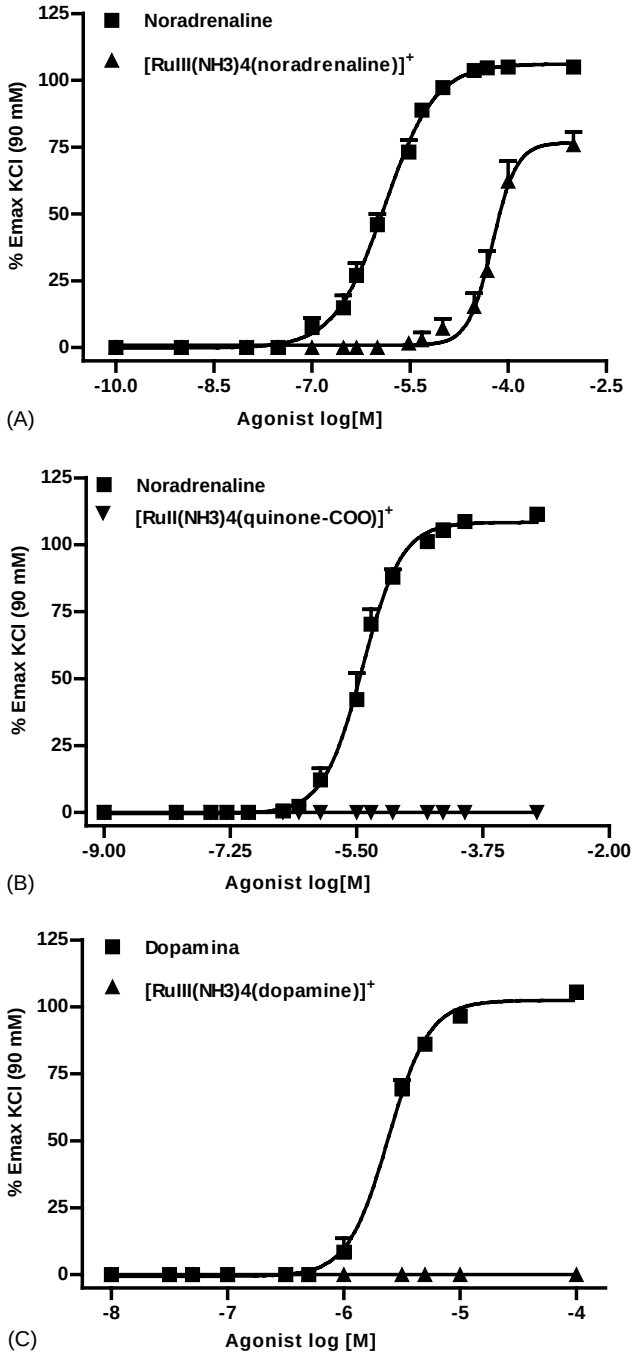


Fig. 8. Effects of $[Ru^{III}(NH_3)_4(catecholamine)]^+$ complexes on isolated rat anococcygeus smooth muscle. Panel (A) shows the effect of introduction of the ruthenium group to noradrenaline molecule. Panel (B) shows the effect of introduction of ruthenium group to noradrenaline in oxidized form. Panel (C) shows the effect of introduction of ruthenium group to dopamine molecule. Data represent the means ± S.E.M. of five to six independent determinations.

4. Conclusions

The present studies establish that the ruthenium–catecholamine complexes are better formulated as $[Ru^{III}(NH_3)_4(catecholamine)]^+$. The existence of this complex underscores the remarkable ability of catecholamine to stabilize the metal ion as Ru(III). The electrochemical and spectroelectrochemical similarities between catecholato complexes and new $[Ru^{III}(NH_3)_4(catecholamine)]^+$ suggest that one electron transfer in those complexes is also likely to happen. Based on the electrochemical behavior, we can conclude that the oxidation of the coordinated catecholamine occurs with a one-electron process followed by a fast inner electron transfer between Ru(III) and the semiquinonato form giving Ru^{II}–quinone. The ruthenium ion reduces the pharmacological activity level of noradrenaline and completely inhibits the pharmacological activity of the dopamine. In the quinone form, the catecholamine complexes do not show any pharmacological activity.

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