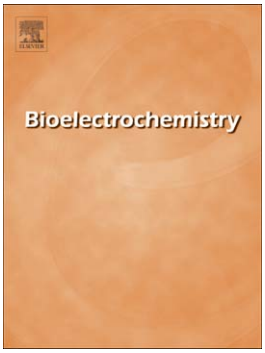

Human Colon Adenocarcinoma HT-29 cell: Electrochemistry and Nicotine Stimulation

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PII: S1567-5394(13)00055-8
DOI: doi: 10.1016/j.bioelechem.2013.05.004
Reference: BIOJEC 6680

To appear in: *Bioelectrochemistry*

Received date: 12 March 2013
Revised date: 14 May 2013
Accepted date: 17 May 2013

Please cite this article as: S.C.B. Oliveira, I.B. Santarino, T.A. Enache, C. Nunes, J. Laranjinha, R.M. Barbosa, A.M. Oliveira-Brett, Human Colon Adenocarcinoma HT-29 cell: Electrochemistry and Nicotine Stimulation, *Bioelectrochemistry* (2013), doi: 10.1016/j.bioelechem.2013.05.004

Human Colon Adenocarcinoma HT-29 cell: Electrochemistry and Nicotine Stimulation

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Abstract

Recently, it was demonstrated that colorectal cancer HT-29 cells can secret epinephrine (adrenaline) in an autocrine manner to auto-stimulate cellular growth by adrenoreceptors activation, and that this secretion is enhanced by nicotine, showing an indirect relation between colorectal cancer and tobacco. The electrochemical behaviour of human colon adenocarcinoma HT-29 cells from a colorectal adenocarcinoma cell line, the hormone and neurotransmitter epinephrine, and nicotine, were investigated by cyclic voltammetry, using indium tin oxide (ITO), glassy carbon (GC) and screen printed carbon (SPC) electrodes. The oxidation of the HT-29 cells, previously grown onto ITO or SPC surfaces, followed an irreversible oxidation process that involved the formation of a main oxidation product that undergoes irreversible reduction, as in the epinephrine oxidation mechanism. The effect of nicotine stimulation of the HT-29 cells was also investigated. Nicotine, at different concentration levels 1, 2 and 15 mM, was introduced in the culture medium and an increase with incubation time, 0 to 3 h and 30 min, of the HT-29 cells oxidation and reduction peaks was observed. The interaction of nicotine with the HT-29 cells stimulated the epinephrine secretion causing an increase in epinephrine release concentration, and enabling the conclusion that epinephrine and nicotine play an important role in the colorectal tumour growth.

Keywords: Colon cancer HT-29 cells, epinephrine, nicotine, carbon electrodes, ITO electrodes.

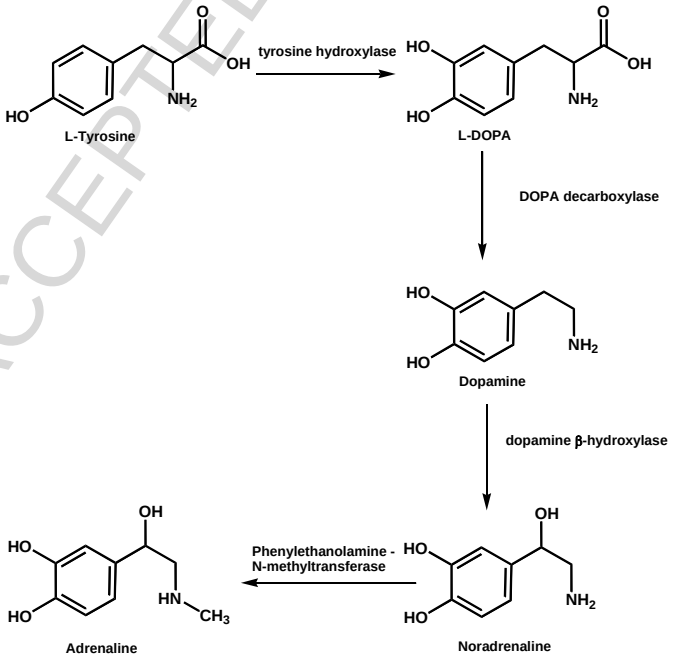
1. Introduction

Colorectal cancer is the third most common cancer in the world [1, 2, 3], and although the management of patients with colorectal cancer has changed dramatically over the past decade, with relevant improvement in survival [4, 5], a significant proportion of patients relapse after a combination of surgery and adjuvant therapies and do not respond to treatment in the metastatic setting [3]. Studies suggested that smokers have a higher risk for developing colorectal cancer cells than non-smokers [6].

Colorectal cancer patients suffer from various forms of psychological stress reflected in elevated plasma catecholamines levels [7], and it has been shown that HT-29 human colon carcinoma cells, as well as other cancer cells, express α7-nicotinic acetylcholine receptor (α7-nAChR), catecholamine-synthesizing enzymes (tyrosine hydroxylase (TH), and DOPA decarboxylase (DDC), dopamine-β-hydroxylase (DβH), phenylethanolamine N-methyltransferase (PNMT) and adrenergic receptors [8, 9, 10].

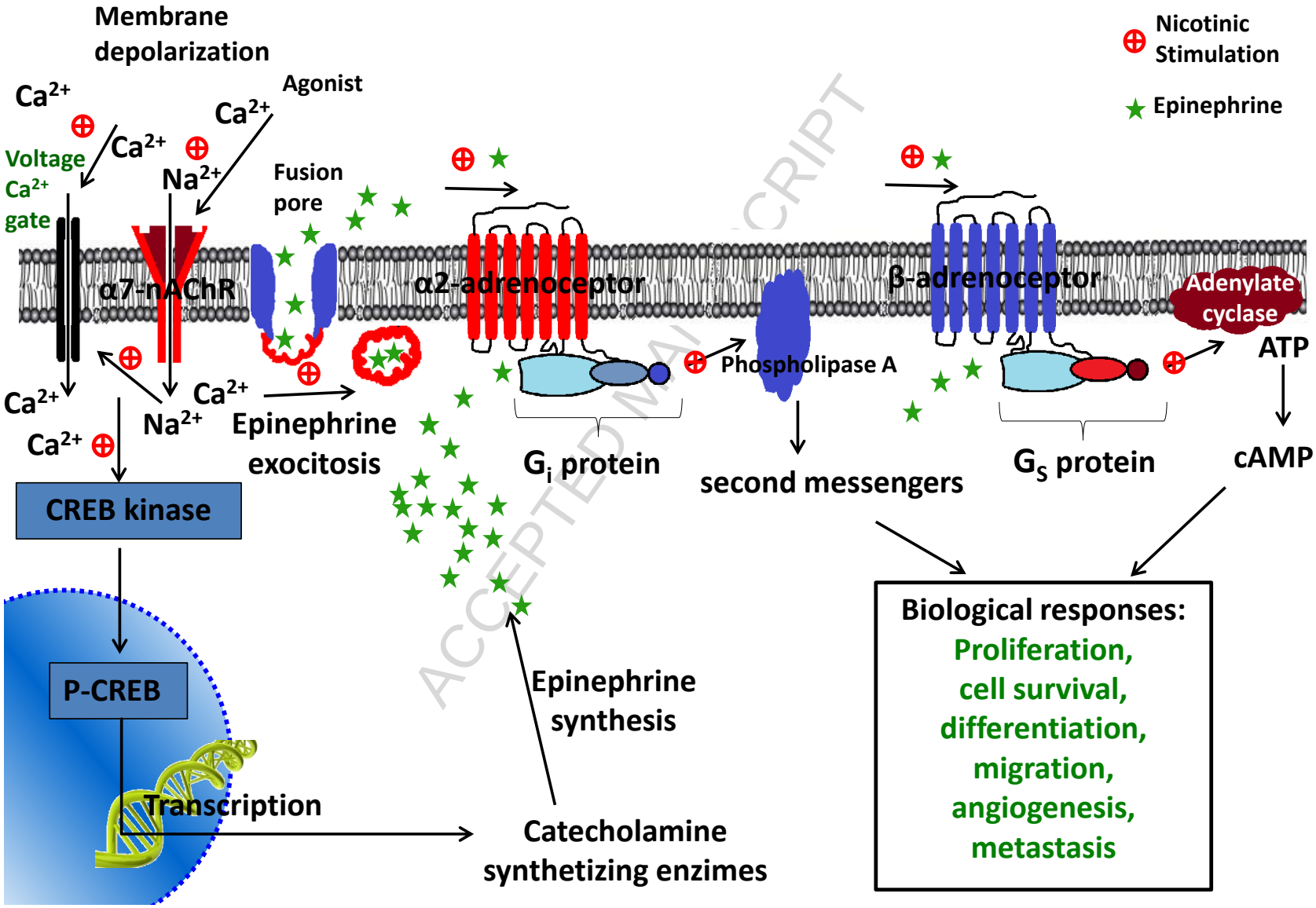
Nicotinic acetylcholine receptors (nAChRs), are ionotropic receptors that form ligand-gated ion channels (Na^+, K^+ and Ca^{2+}) in the plasma membranes of certain cells.

The catecholamine-synthesizing enzymes are important in the biosynthesis of epinephrine, [11], **Scheme 1.** Through the activated ionic channels or due to the membrane depolarization, Ca^{2+} can enter the cells and favour activation (*i.e.*, phosphorylation) of the transcription factor CREB (cAMP response element-binding protein), which in turn modifies gene expression [10, 12, 13].



Scheme 1. Epinephrine synthesis mechanism [11].

As a particular case, previous studies on normal and cancer cells have suggested that nicotine, a major active component of cigarette smoke, can regulate the expression of catecholamine-synthesis pathway through the action on α7-nAChR, epinephrine production and ultimately epinephrine release and adrenergic receptors activation [6, 13, 14], **Schemes 2 and 3**.



Scheme 2. Chemotherapy resistance and cell proliferation promoted by epinephrine via nAChR activated by nicotine.

The α2-adrenoceptor is a transmembrane G protein-coupled receptor (GPCR) associated with the G_i heterotrimeric G-protein. When the α2-adrenoceptor is activated by epinephrine, G_i is released and inhibits the cAMP dependent pathway by inhibiting adenylate cyclase activity, decreasing the production of cAMP from ATP, which, in turn, results in a decreased activity of cAMP-dependent protein kinase [15]. The phospholipases pathway can also be activated by G_i. Enzymatic processing of phospholipids by phospholipases convert these molecules into lipid mediators or second messengers (such as arachidonic acid, phosphatidate and diacylglycerol) that play key roles in membrane trafficking, signal transduction, cell proliferation and tumour progression [16].

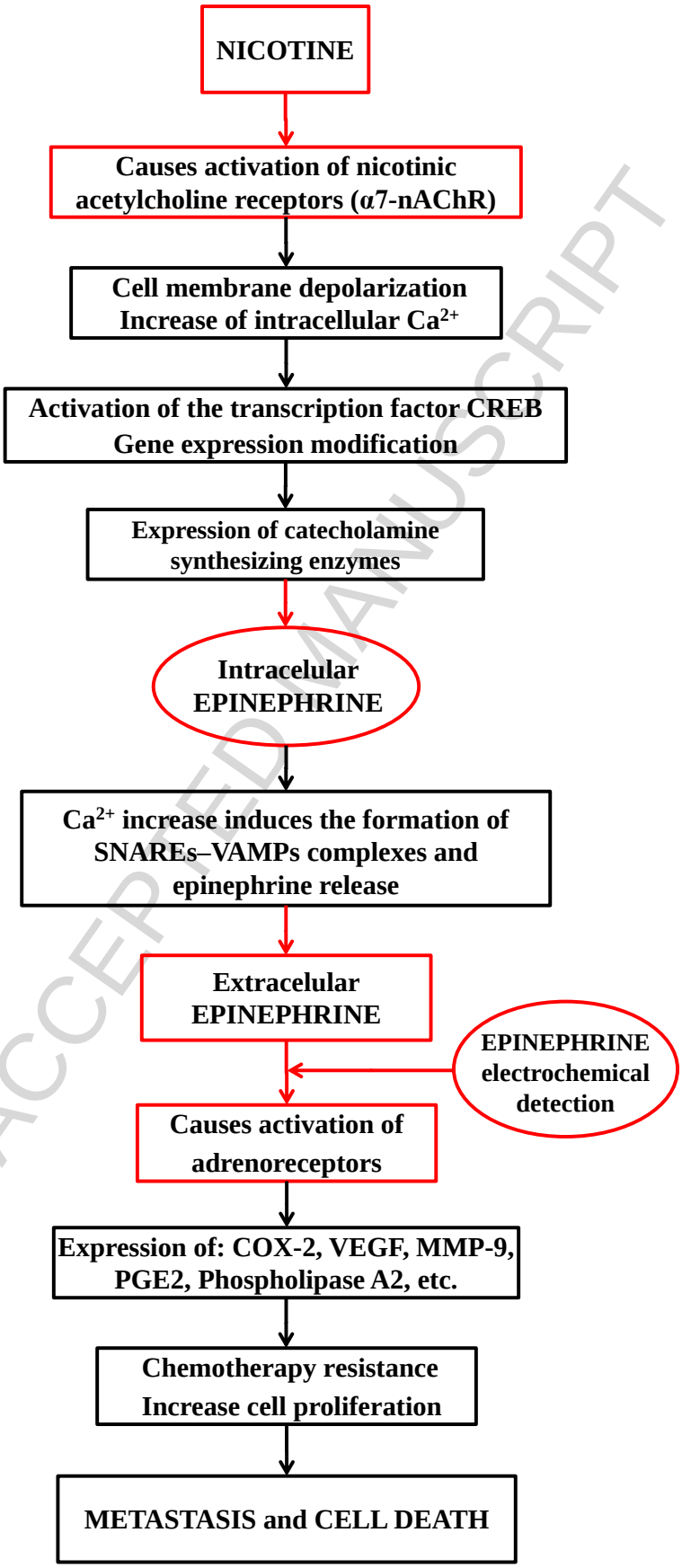
Moreover, it was demonstrated that stimulation of β-adrenoceptors by epinephrine play a role in the HT-29 cell proliferation in a dose-dependent manner and enhance prostaglandin-endoperoxide synthase 2 **(**COX-2) and vascular endothelial growth factor (VEGF) expression [6, 8, 17, 18]. Epinephrine at the concentration of 10 μM significantly increased HT-29 cell proliferation by 35% when compared to the control group and also up-regulated matrix metallopeptidase 9 **(**MMP-9) activity and prostaglandin E2 (PGE2) release. [6, 18, 19].

All these effects induced by epinephrine on the physiology of cancer cells lead to poor response of tumours to chemotherapy, and constitute a poor prognostic factor for survival [18, 19].

Epinephrine has four polar functional groups and a small carbon skeleton. Thus, the polar functional groups dominate in determining the character of the molecule making it very polar and unlikely to pass through the cell membrane. In aqueous solution, the four polar groups will be highly solvated with water molecules. In order to cross the cell membrane, these water molecules have to be "stripped away" and this involves energy [20]. Following an appropriate cell stimulation (which provokes a Ca^{2+} entry or increase) and multiple protein-protein interactions, that is, the formation of soluble *N*-ethylmaleimide sensitive fusion protein attachment receptors (SNAREs) - vesicle-associated membrane proteins (VAMPs) complexes, the intracellular epinephrine content is released to the extracellular medium [21, 22, 23].

Considering the existence of vesicle-associated membrane proteins (VAMPs) in HT-29 cells, as well as in other non-neuroendocrine cells [24, 25, 26], and the fact that the activation of nAChRs by nicotine results in the influx of Na^+ and Ca^{2+}, which engenders rapid changes in the membrane potential and local increase in intracellular Ca^{2+} concentration [17], it is explained the epinephrine release by HT-29 cells by a similar mechanism, in which nicotine play a pivotal role in chemotherapy resistance and cell proliferation [6, 13, 14].

The HT-29 colon cancer cells can produce epinephrine via catecholamine-synthesizing enzymes (TH, DDC, DβH and PNMT) and nicotine can stimulate this process as well as epinephrine release. However, the expression of catecholamine-synthesizing enzymes and epinephrine release in the extracellular medium onsets depends on the availability of intracellular Ca^{2+} but the composition of the external environment (pH, nature and concentration of ions, etc.) also plays a role. More generally, on a physicochemical point of view, the secretion and release efficiency and dynamics are finely regulated by the membrane properties (phospholipidic composition, mechanical tension, electrical potential, etc.) [27, 28]. The electrical properties of cell membrane are dependent on all the physical mechanisms, which control the mobility and availability of the relevant ions such as sodium, chloride, potassium, magnesium and calcium [29].



Scheme 3. Nicotine activation of acetylcholine receptors and electrochemical detection of epinephrine secretion.

In order to overtake this harmful effects and cancer progression, suitable procedures are required for monitoring epinephrine secretion. Several procedures based on quantitative gene expression analysis have already been developed and described [6, 8, 9, 10, 13, 17, 24, 30, 31, 32]. However, these methods are expensive, time consuming and require skilled operators.

The objective of this work is to investigate the epinephrine secretion of HT-29 colon cancer cells. The electrochemical methodologies are non-morphological methods for following cell health state and to evaluate the effectiveness of drugs or chemical compounds. Moreover, the electrochemical techniques are recognized experimental tools for detection of cell exocytosis electroactive compounds [32, 33].

The electrochemical behaviour of human colon adenocarcinoma HT-29 cells from a colorectal adenocarcinoma cell line, the hormone and neurotransmitter epinephrine, and nicotine, were investigated by cyclic voltammetry (CV) using indium tin oxide (ITO), glassy carbon (GC) and screen printed carbon (SPC) electrodes. The results obtained enabled the confirmation that HT-29 can secret epinephrine in an autocrine manner. The effect of nicotine stimulation on epinephrine secretion and in HT-29 cell proliferation was also investigated electrochemically following the changes in the electrochemical behaviour of HT-29 cells after different incubation times with nicotine in the cell medium.

2. Experimental

2.1. Materials and reagents

Dulbecco's Modified Eagle Medium (DMEM) with Glutamax and foetal bovine serum (FBS) formulation (pH = 7.0) were purchased from Gibco-Invitrogen. HT-29 cell line, epinephrine and nicotine were purchased from Sigma-Aldrich. Stock solutions of 1.0 mM epinephrine and 62 mM nicotine in deionised water were prepared and diluted to the desired concentration. The other supporting electrolyte solution used was 0.1 M phosphate buffer pH = 7.0. All solutions were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system (conductivity ≤ 0.1 $\mu S\ cm^{-1}$).

Microvolumes were measured using EP-10 and EP-100 Plus Motorized Microliter Pippettes (Rainin Instrument Co. Inc., Woburn, USA). The pH measurements were carried out with a Crison micropH 2001 pH-meter with an Ingold combined glass electrode.

All experiments were done at room temperature, T = 298 K (25 ºC).

2.2. Cell culture

The human colon cancer cell line HT-29 was routinely grown in DMEM medium with FBS (10 % vol/vol), and no antibiotics supplements, onto 75 cm^2 flasks. Cells were maintained at 37 ºC, in a humidified atmosphere of 5 % CO_2. For the experiments, cells were plated on ITO and SPC working electrodes that were deposited on the surface of cell culture dishes until a confluent monolayer was reached (~ 48 h).

2.3. Voltammetric parameters and electrochemical cells

Voltammetric experiments were carried out using an IVIUM potentiostat in combination with IviumSoft program version 1.84 (Ivium Technologies, Eindhoven, The Netherlands). Measurements were carried out using GC (0.0176 cm^2), SPC (0.07 cm^2) and ITO (~ 2 cm^2) as working electrodes with a Pt wire counter electrode, and an Ag/AgCl (3 M KCl) electrode as reference, in a one-compartment electrochemical cell

of 2 mL capacity or directly in the cell culture dishes. The experimental conditions for cyclic voltammetry (CV) were scan rate 50 and 100 mV/s.

2.4. Electrode surface conditioning

Different procedures for GC, SPC and ITO surface pre-treatments were carried out before every electrochemical assay, ensuring very reproducible experimental results.

The GC electrode was polished using diamond spray (particle size 3 μm (Kement, Kent, UK), before every electrochemical assay. After polishing, the electrode was rinsed thoroughly with Milli-Q water. Following this mechanical treatment, the GC electrode
was placed in phosphate buffer supporting electrolyte and CVs were recorded starting at 0.0 V initial potential in the positive going direction, in the potential range - 0.5 V to + 1.2 V, for 5 cycles, scan rate 50 mV/s.

The SPC electrode was pre-treated anodically by potential cycling from 0.0 V to + 1.2 V, for 10 cycles, scan rate 100 mV/s, before every electrochemical assay in phosphate buffer.

The ITO electrode was electrochemically pre-treated by potential cycling, starting at 0.0 V initial potential in the positive going direction, in the potential range - 0.5 V to + 0.8 V, for 5 cycles, scan rate 100 mV/s, before every electrochemical assay in phosphate buffer.

3. Results and discussions

3.1. Electrochemical behaviour of epinephrine and nicotine

The electrochemical redox mechanism of epinephrine and nicotine was investigated by CV at GC, SPC and ITO electrodes in 0.1 M phosphate buffer pH = 7.0 and in Dulbecco's Modified Eagle's Medium (DMEM) formulation (pH = 7.0). This study was a control to enable the detection of the epinephrine release by HT-29 colon cancer cells and the nicotine stimulation effect.

The electrochemical behaviour of DMEM supporting electrolyte was investigated by CV at GC, SPC and ITO electrodes, and showed that DMEM is an inert electrolyte,
Fig. 1.

Here Fig. 1.

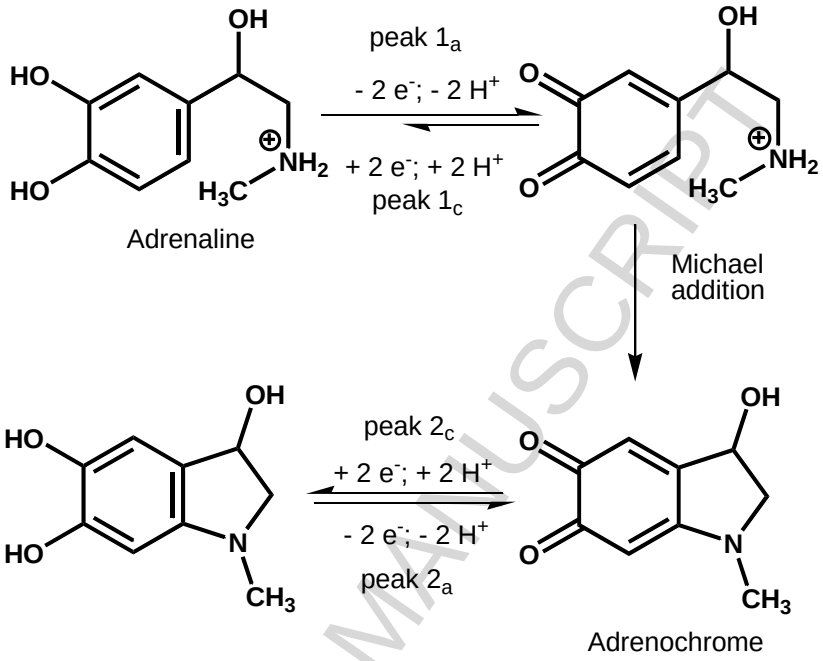
3.1.1. Epinephrine

The electrochemical behaviour of 250 μM epinephrine at a GC electrode by CV in phosphate buffer pH = 7.0 and in DMEM was the same, **Fig. 2**. On the positive going scan the oxidation peak 1a, at E_{p1a} = + 0.30 V, corresponds to the oxidation of the catechol moiety to *ortho*-quinone. Reversing the scan direction occurred a small cathodic peak 1c, at E_{p1c} = + 0.16 V, followed by the reversible 2c-2a redox couple, at E_{p2c} = - 0.18 V and E_{p2a} = - 0.15 V.

Here Fig. 2.

Epinephrine redox mechanism in physiological pH is coupled with a chemical reaction (Michael addition) which follows the electrochemical oxidation of the catechol moiety to *ortho*-quinone and results in cyclisation of epinephrine oxidation product and

adrenochrome formation, which undergoes reduction at negative potentials [34, 35], **Scheme 4**.



Scheme 4. Epinephrine oxidation mechanism at physiological pH [34, 35].

The electrochemical behaviour, using CV and an ITO electrode, in 500 µM epinephrine in phosphate buffer pH = 7.0, and in saturated epinephrine in DMEM is a little different from the results obtained with the GC electrode, **Fig. 3**. On the positive going scan, potential range 0.0 V till + 0.5 V, the oxidation peak 1a, at E_{p1a} = + 0.38 V, corresponding to the oxidation of the catechol moiety to *ortho*-quinone was observed. Reversing the scan direction, from + 0.5 V till - 0.5 V, only occurs the cathodic peak 2c, at E_{p2c} = - 0.38 V, **Fig. 3**, corresponding to the reduction of adrenochrome formed after the epinephrine oxidation, **Scheme 3**.

Here Fig. 3.

Higher peak currents were obtained in the epinephrine saturated solution and a decrease of the oxidation peak 1a of epinephrine was observed, whereas the peak 2c remains constant with increasing the number of scans, **Fig. 4.** The electrochemical behaviour of 500 µM epinephrine at a SPC electrode in phosphate buffer pH = 7.0 and in DMEM, showed the same behaviour when compared with the results obtained at ITO electrode. An irreversible anodic peak 1a, at E_{p1a} = + 0.40 V, and after reversing the scan direction till - 0.5 V the cathodic peak 2c, at E_{p2c} = - 0.59 V, was detected.

Here Fig. 4.

The ITO and SPC electrodes were used in all experiments with the HT-29 cells due to their simplicity and flexibility to be introduced in the cell culture dishes and also enabling the cell growth on their electrode surface.

3.1.2. Nicotine

The electrochemical behaviour of 15 mM nicotine was investigated using SPC and ITO electrodes in phosphate buffer pH = 7.0 and in DMEM, starting at an initial potential of 0.0 V, scanning in the positive direction to + 0.8 V, and after reversing the scan in the negative potential limit of - 0.5 V, at scan rate 100 mV/s. No oxidation or reduction peaks were detected showing that, in the experimental conditions used, nicotine is not electroactive on SPC and ITO electrodes [36, 37]

3.2. Electrochemical behaviour of HT-29 cells

The HT-29 cells in DMEM were growth onto ITO and SPC electrode surfaces.

The first CV of HT-29 cells grown on the ITO electrode surface in DMEM, was recorded in the potential range 0.00 V till + 0.80 V, at 50 mV/s, **Fig. 5A**. Successive CVs showed only the irreversible anodic peak 1a, at E_{p1a} = + 0.42 V, and the peak 1a current decreased due to the adsorption of the oxidation products at the ITO electrode surface, **Fig. 5A**.

Successive CVs were also recorded in the same HT-29 cells sample, starting at the 0.0 V initial potential, scanning in the positive direction to + 0.8 V and after reversing the scan to the negative potential of - 0.5 V, **Fig. 5B**. The CV showed in the first scan only the cathodic peak 2c, at E_{p2c} = - 0.38 V, **Fig. 5B**, followed by the anodic peak 1a, at E_{p1a} = + 0.42 V.

Here Fig. 5.

In other experiments, successive CVs for new HT-29 cells grown onto ITO electrode surface in DMEM were recorded following two steps. The first CV was recorded only in the cathodic region between the potentials 0.0 V to - 0.5 V, and no reduction peak was detected. The second CV in the same HT-29 cells was recorded in the potential range - 0.5 V to + 0.8 V, starting at 0.0 V initial potential in the positive going direction.

The CVs, **Fig. 6**, showed the same behaviour as epinephrine at ITO electrode, **Fig. 4**. On the first anodic scan, one small oxidation peak 1a, at E_{p1a} = + 0.25 V, followed, in the negative going scan, by the reduction peak 2c, at E_{p2c} = - 0.3 V, **Fig. 6**.

Here Fig. 6.

In successive CVs, **Fig. 6,** the anodic and cathodic peak currents increased and the peak potentials were slightly shifted to more positive and negative potentials, respectively, and, when epinephrine was added to the HT-29 cells culture, an increase of HT-29 peak current was observed.

The epinephrine adsorption onto ITO electrode during the oxidation of HT-29 cells was also investigated. After recording several CVs of HT-29 cells grown onto ITO electrode surface, between the potential limits + 0.8 V till - 0.5 V in DMEM, the HT-29 cells were removed and the electrode was washed and then transferred to DMEM. On the first, positive going scan, no oxidation peak appeared; nevertheless reversing the scan direction the epinephrine reduction peak 2c, at E_{p2c} = - 0.35 V, occurred, showing that after the HT-29 cells were removed and the ITO electrode was washed only the adsorbed oxidized epinephrine product remained on the electrode surface. Reversing the scan direction, oxidation peak 1a, at E_{p1a} = + 0.3 V, occurred.

Similar experiments were performed using SPC electrodes, **Fig. 7**.

Successive CVs were recorded for HT-29 cells grown onto the SPC electrode surface in DMEM, at 50 mV/s, using three different potential windows: (a) 0.0 V to - 0.5 V, (b) 0.0 V to + 0.8 V and (c) + 0.8 V to - 0.8 V.

Using potential window (a) no reduction process was detected, using potential window (b) the CVs showed, as expected, the irreversible anodic peak 1a, at E_{p1a} = + 0.40 V and peak 1a current decreased in the successive scans, **Fig. 7A**. Using potential window (c) the CVs showed the anodic peak 1a, at E_{p1a} = + 0.40 V, and the cathodic peak 2c, at E_{p2c} = - 0.64 V.

Here Fig. 7.

The electrochemical behaviour of HT-29 cells on SPC electrode, **Fig. 7**, and on ITO electrode is the same, **Fig. 6**, and equal to the electrochemical behaviour of epinephrine on ITO and SCP electrodes, **Figs. 3 and 4**.

Based on the results obtained with HT-29 cells, **Figs. 5, 6 and 7**, and in agreement with the electrochemical behaviour of epinephrine control on the ITO and SPC electrode surface, **Figs. 3 and 4**, the peak 1a corresponds to the oxidation of the epinephrine secreted by the HT-29 cells, **Scheme 3**.

Living cells have a negative membrane potential ranging from – 100 mV for normal cells to very low still negative potential, for cancer cells. This negative potential indicates that the inside surface of the cell membrane is relatively more negative than the immediate exterior surface [29]. Cell receptors can be activated by electric fields [29], and signalling molecules such as Ca^{2+} can enter inside the cell initiating epinephrine release.

When positive potentials were applied to the HT-29 cells grown on the ITO, first anodic scan, the HT-29 cells membrane potential was dramatically changed. In this way, in the first positive scan epinephrine was released and oxidized. Reversing the scan direction the epinephrine oxidation product adrenochrome reduction peak 2c, is observed, **Fig. 6**, and peak 1a and 2c currents increased in the successive scans, **Figs. 5, 6 and 7.**

The electrochemical behaviour of the human colon HT-29 cells grown on ITO and SPC electrode surface showed that epinephrine secretion play an important role in the colorectal tumour growth, and the effect of nicotine stimulation of epinephrine secretion and HT-29 cell proliferation was also investigated. The effect of the nicotine interaction with HT-29 cells was followed comparing the changes in the absence/presence of nicotine on the HT-29 cells electrochemical behaviour.

The HT-29 cells were grown onto the ITO and SPC electrode surfaces and incubated for different time periods in DMEM containing 1, 2 and 15 mM nicotine. The interaction between 1, 2 and 15 mM nicotine and the HT-29 cells was first studied after short 30 min and 1 h incubation times, and no difference was detected on the HT-29 cells redox behaviour when compared with the HT-29 cells in the absence of nicotine.

Increasing the incubation time for 3 h 30 min, between 15 mM nicotine and the HT-29 cells, the CVs showed an increase of peak 1a and 2c currents, indicating that after a long incubation time nicotine promoted the increase of the epinephrine secreted by the HT-29 cells, **Fig. 8.**

Here Fig. 8.

The electrochemical results showed that during the colon cancer HT-29 cell culture proliferation on ITO and SPC electrode surfaces epinephrine was secreted in an

autocrine manner to self-stimulate cell growth via adrenoceptors, and the secretion was increased by the presence of nicotine via the up-regulation of the expression of the catecholamine-synthesizing enzymes TH, AADC, DßH and PNMT.

4. Conclusions

The redox behaviour of epinephrine at GC, SPC and ITO electrodes was investigated in 0.1 M phosphate buffer pH = 7.0 and DMEM, showing a complex mechanism involving the oxidation of the catechol group followed by intra-cyclization via Michael addition and the formation of reducible adrenochrome.

The electrochemical study showed that colon cancer HT-29 cells secretion undergo oxidation on ITO and SPC electrodes, and was confirmed that epinephrine was secreted in an autocrine manner by the HT-29 cells. During the cellular division the catecholamine-synthesizing enzymes are expressed and produce epinephrine that, together with the applied potential to the electrode surface, favours the intracellular epinephrine to be released to the extracellular medium, enabling its electrochemical detection.

The epinephrine secretion and release processes were stimulated by the presence of different concentration of nicotine, enabling the conclusion that epinephrine and nicotine play an important role in colorectal tumour growth. These findings are important in understanding the pathogenesis of colon cancer, especially related to stress, and can bring progresses for new non-invasive methods in development of specific inhibitors of colorectal cancer cell proliferation.

Acknowledgements

Financial support from Fundação para a Ciência e Tecnologia (FCT), Post-Doctoral Grant SFRH/BPD/71965/2010 (S.C.B. Oliveira), SFRH/BPD/80195/2011 (T. A. Enache) and SFRH/BPD/46149/2008 (C. Nunes), BTID Grant (I.B. Santarino), projects PTDC/QUI/098562/2008, PTDC/SAU-NEU/103538/2008, POPH (co-financed by the European Community Funds FSE e FEDER/COMPETE), CEMUC-R (Research Unit 285), is gratefully acknowledged.

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Figure Captions

Fig 1. CVs in supporting electrolyte (▬) pH = 7.0 and (▪▪▪) DMEM on: (**A**) ITO (**B**) SPC.

Fig 2. CVs of epinephrine at GC electrode: (**A**) [epinephrine] = 250 µM at pH = 7.0 in [phosphate buffer] = 0.1 M and (**B**) [epinephrine] = 250 µM in DMEM: (▬) first scan, (▪▪▪) second scan, and (▬) supporting electrolyte,ν = 50 mV s^{-1}.

Fig 3. CVs of epinephrine at ITO electrode: (**A**) [epinephrine] = 500 µM at pH = 7.0 in [phosphate buffer] = 0.1 M and (**B**) [epinephrine] = 500 µM at DMEM: (▬) first scan, and (▬) supporting electrolyte, ν = 50 mV s^{-1}.

Fig. 4. CVs in DMEM of a saturated epinephrine solution at ITO electrode: (▬) first scan, and (▬) successive scans, ν = 50 mV s^{-1}.

Fig. 5. Successive CVs in DMEM of HT-29 cells on ITO electrode: (**A**) E_1= 0.0 V, E_2 = + 0.8 V and E_3 = 0.0 V, (**B**) E_1 = 0.0 V, E_2 = +0.8 V and E_3 = - 0.5 V; E_1 – initial potential, E_2 – maximum potential and E_3 – minimum potential. (▬) first scan, and (▬) successive scans, ν = 50 mV s^{-1}.

Fig. 6. Successive CVs in DMEM of HT-29 cells on ITO electrode, ν = 50 mV s^{-1}.

Fig. 7. Successive CVs in DMEM of HT-29 cells on SPC electrode: (**A**) E_1 = 0.0 V, E_2 = + 0.8 V and E_3 = 0.0 V, (**B**) E_1 = 0.0 V, E_2 = +0.8 V and E_3 = - 0.8 V; E_1 – initial potential, E_2 – maximum potential and E_3 – minimum potential. (▬) first scan, and (▬) successive scans, ν = 50 mV s^{-1}.

Fig. 8. CVs in DMEM of nicotine stimulated HT-29 cells on ITO electrode: (▬) nicotine stimulated HT-29 cells, 3 h 30 min incubation time between HT-29 cells and [nicotine] = 15 mM, and (▬) HT-29 cells control, ν = 50 mV s^{-1}.

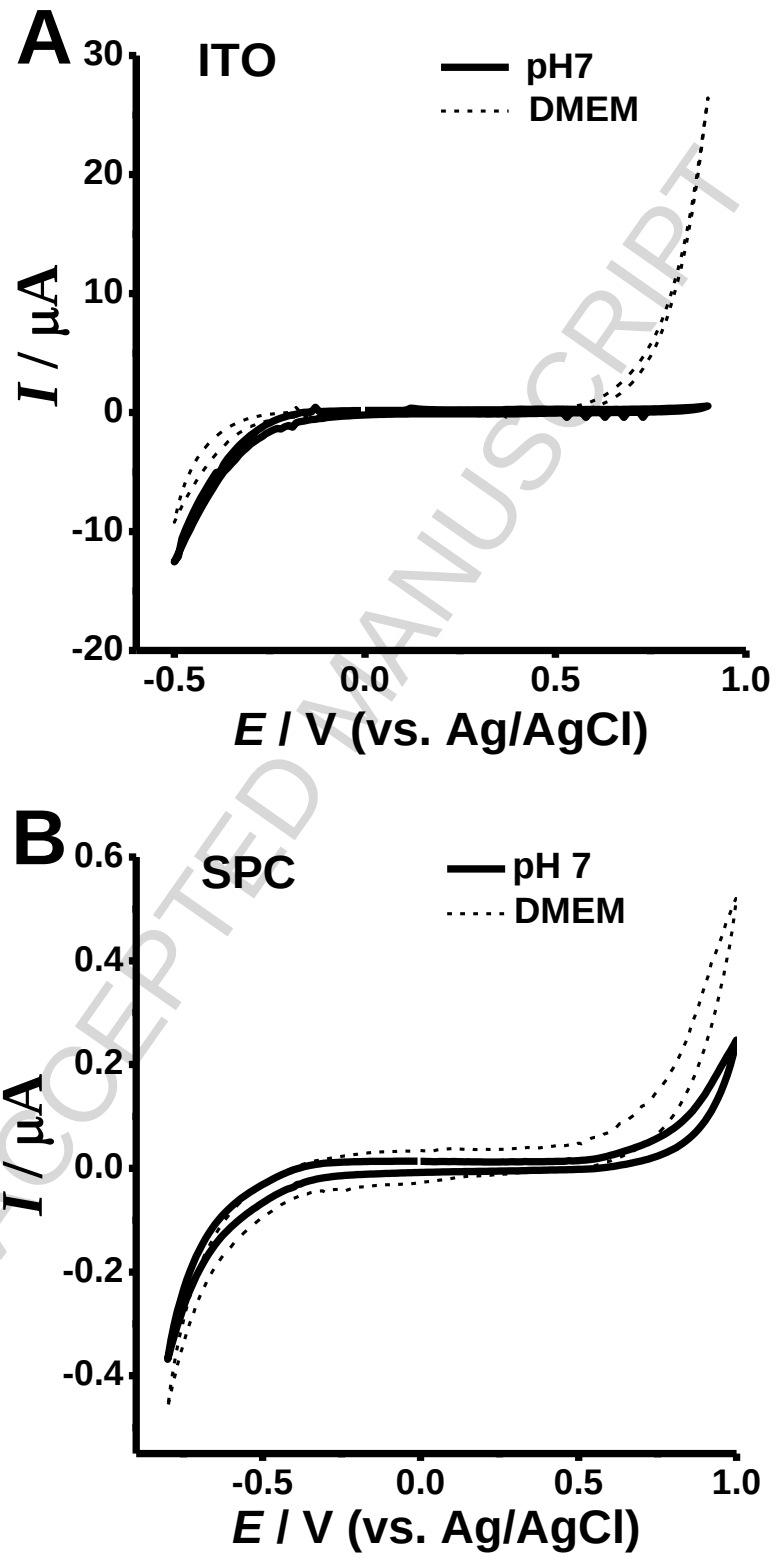


Fig 1.

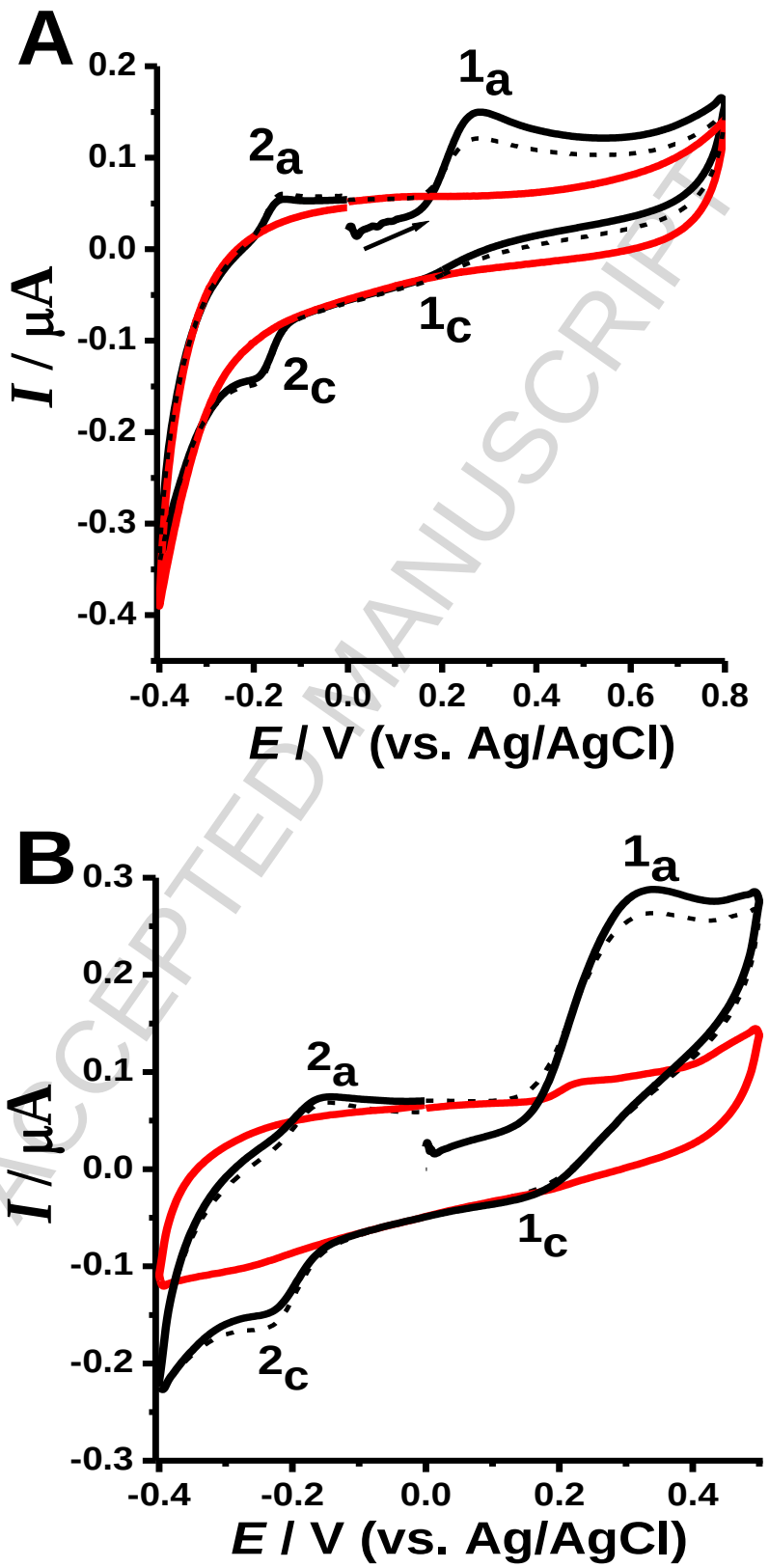


Fig 2.

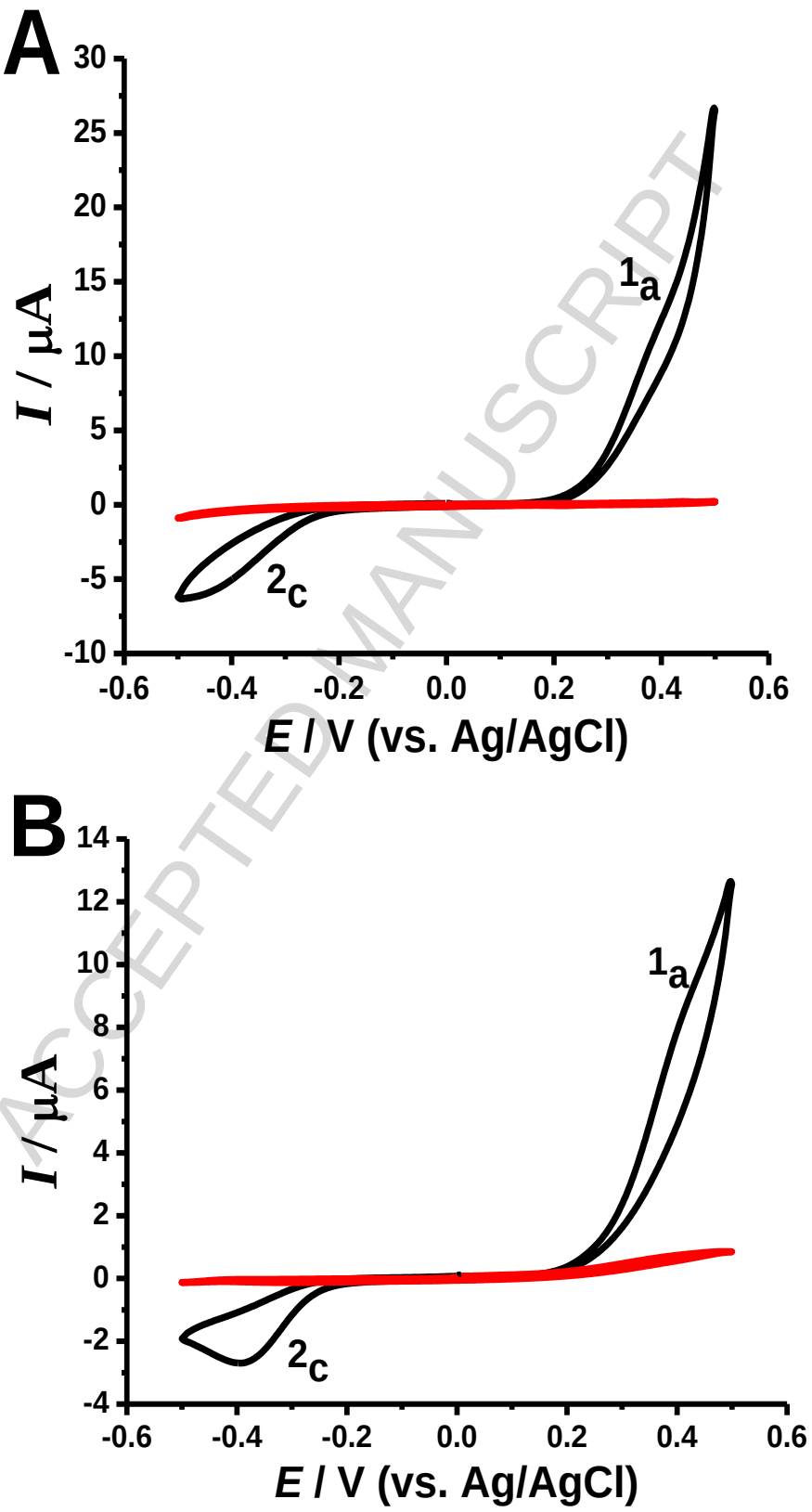


Fig 3.

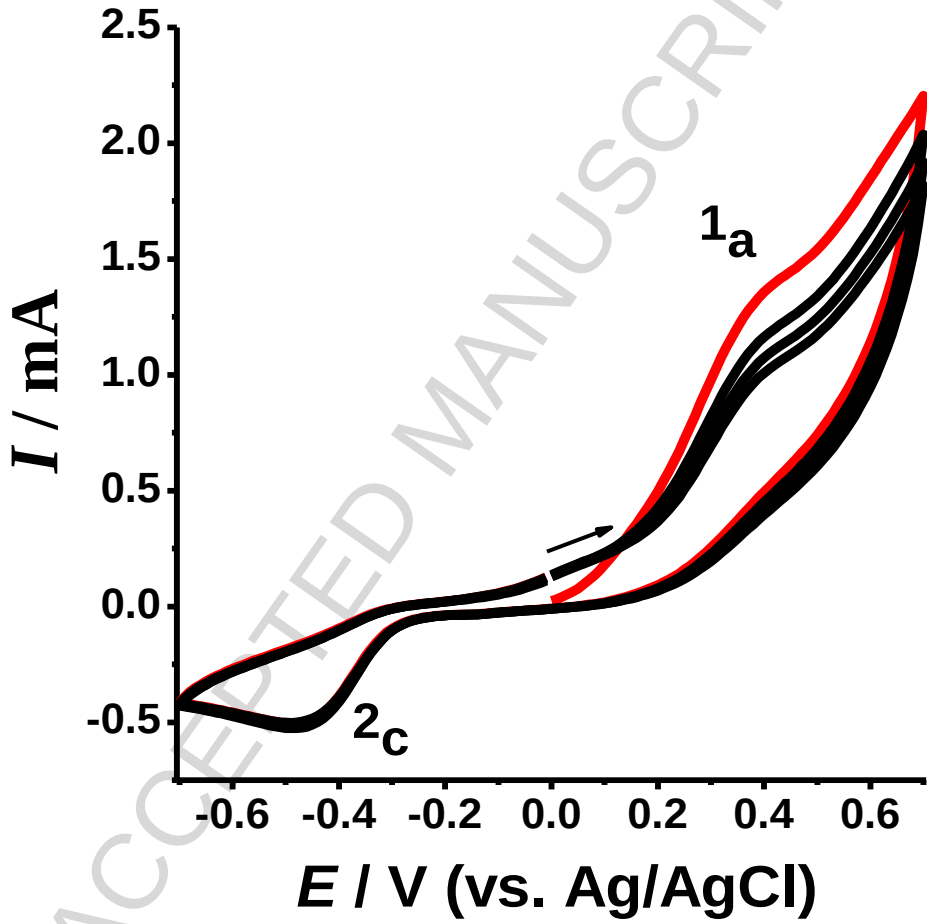


Fig. 4.

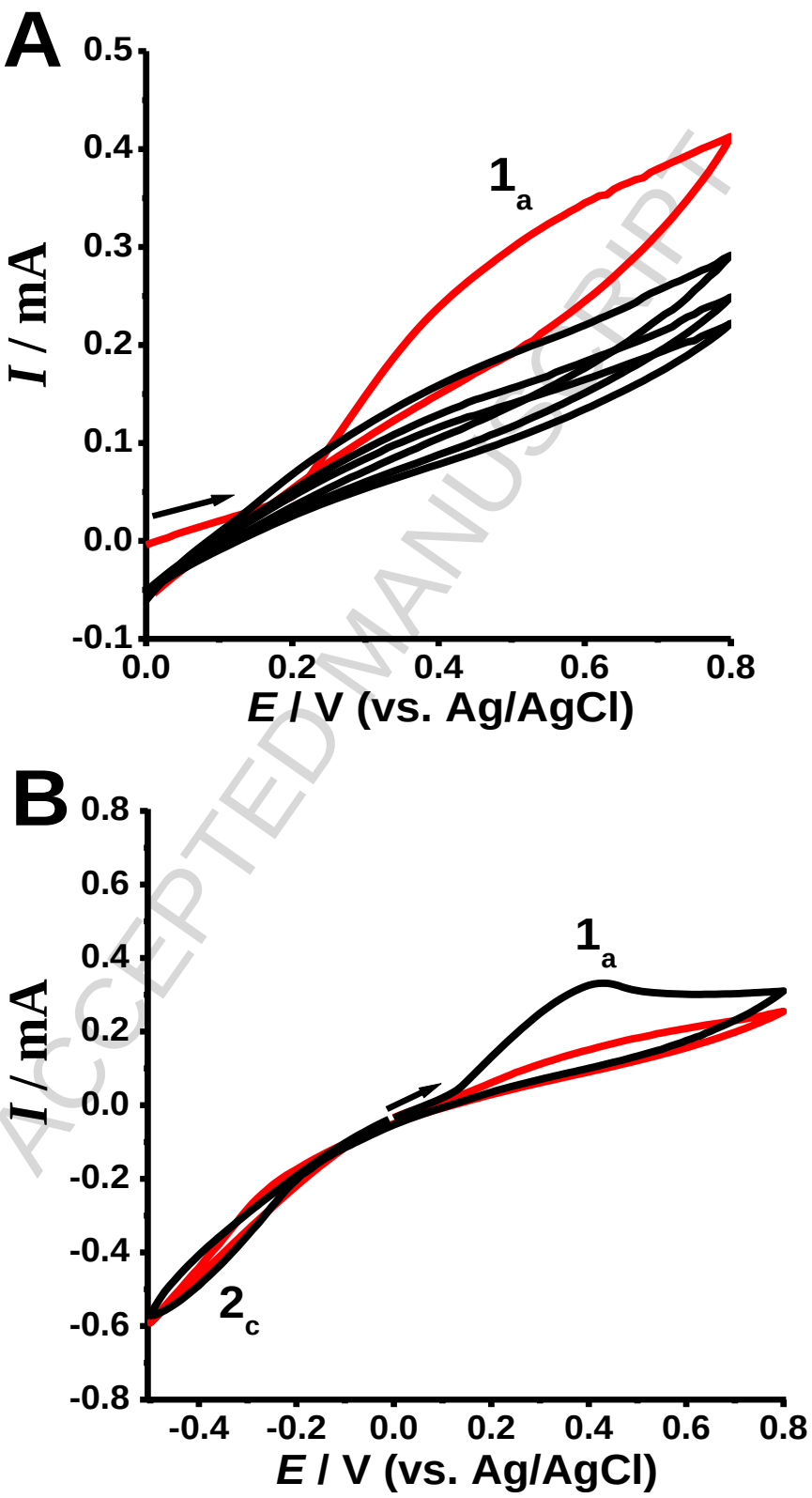


Fig. 5.

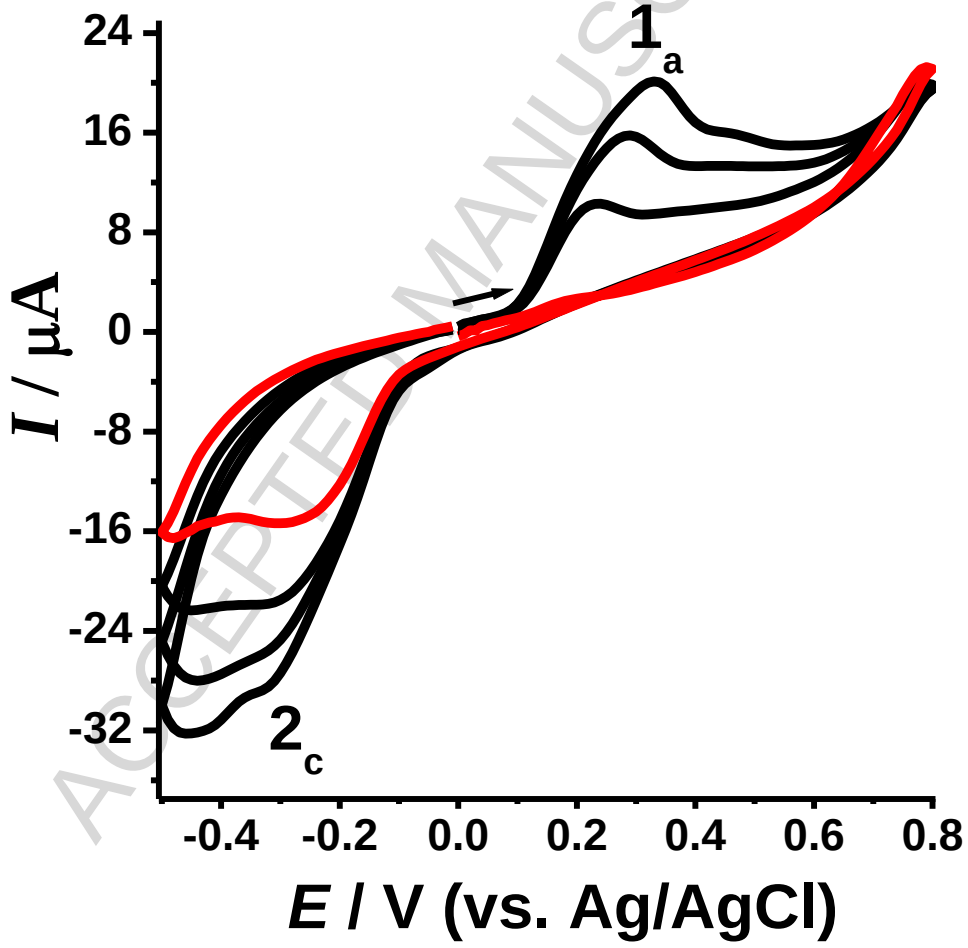


Fig. 6.

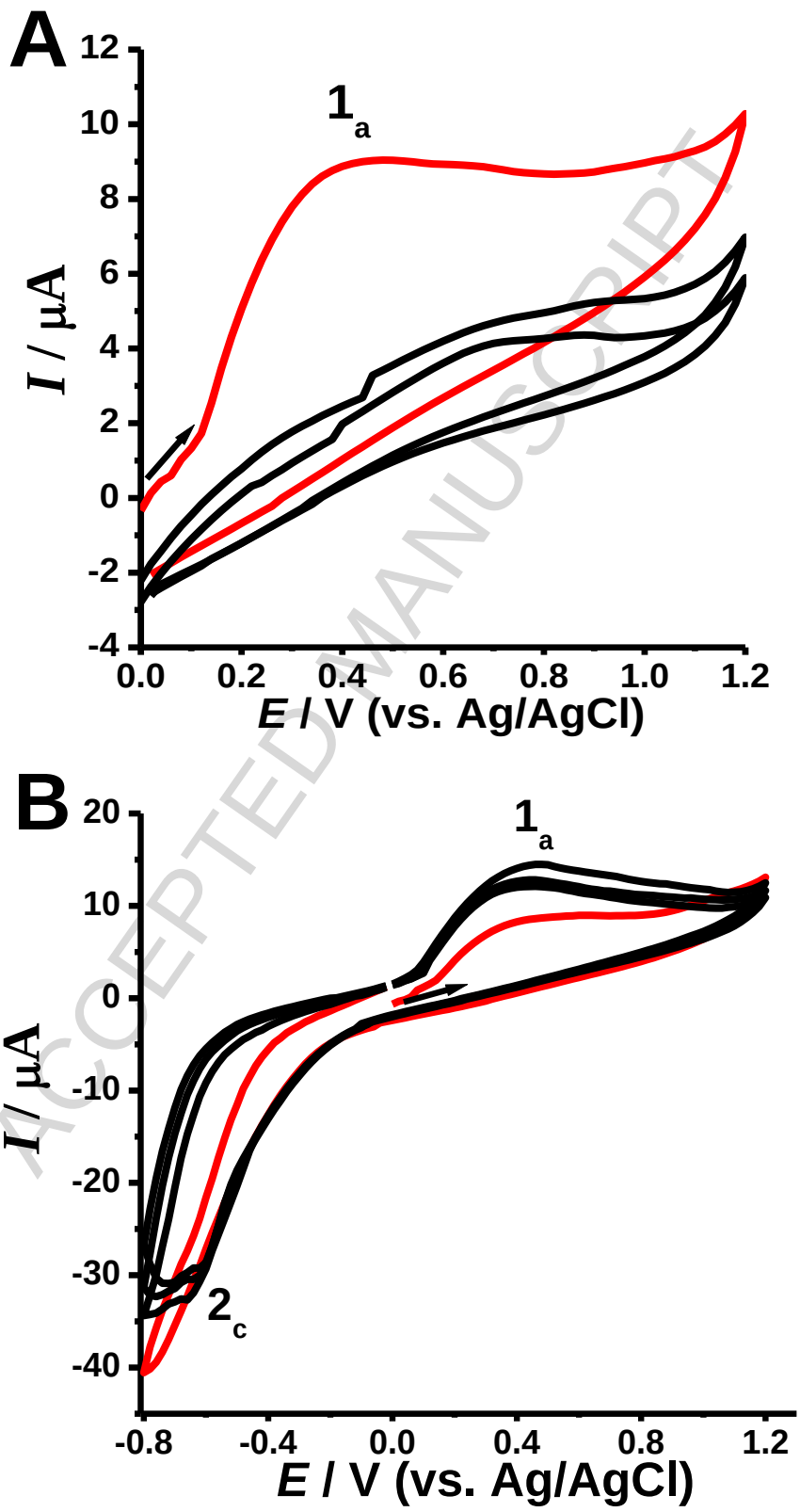


Fig. 7.

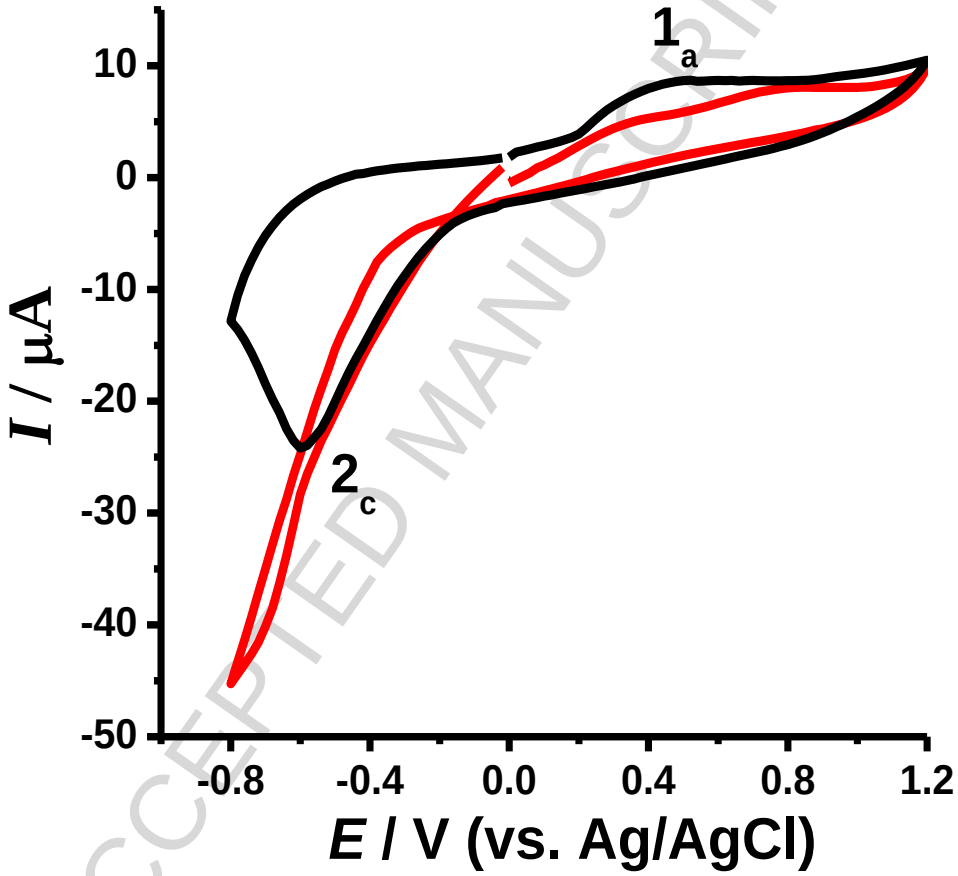
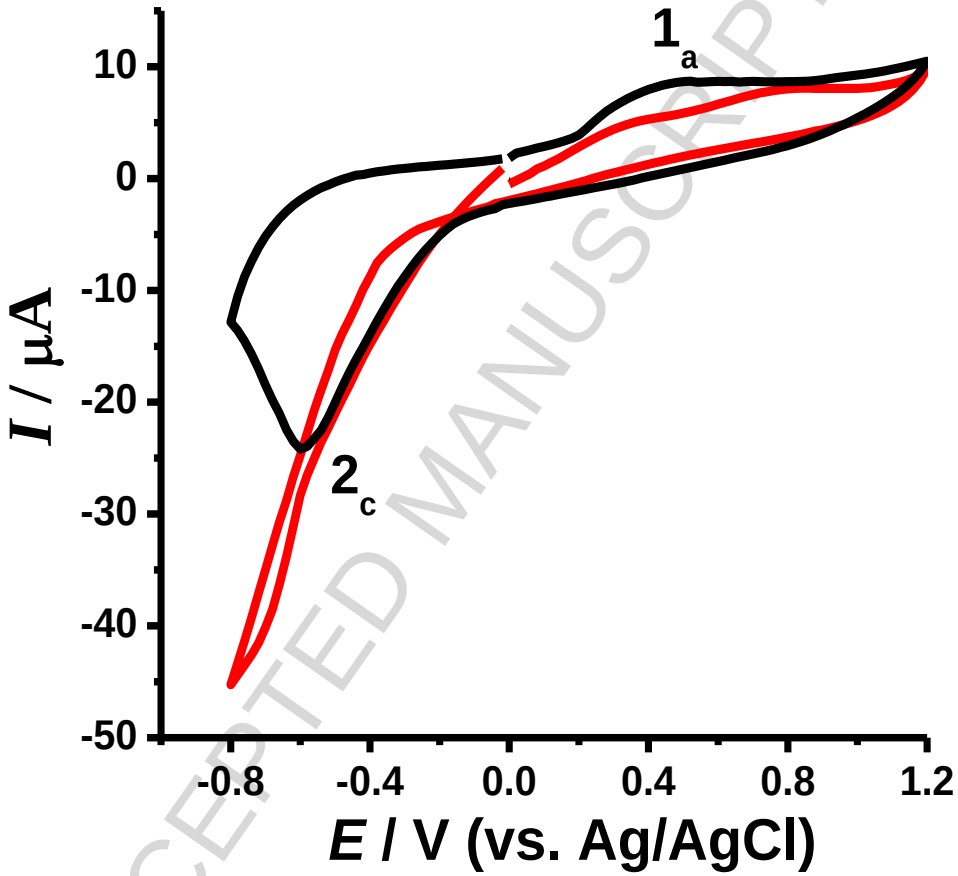


Fig. 8.

Graphical Abstract



Highlights

- The electrochemical behaviour of human colon HT-29 cells was investigated.
- The HT-29 cells undergo oxidation on ITO and SPC electrodes.
- The oxidation is associated with the epinephrine secreted by the HT-29 cells.
- The epinephrine secretion and release were stimulated by the presence of nicotine.
- Epinephrine and nicotine play an important role in colorectal tumour growth.