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Evaluation of pH influence on in vitro permeation of salicylic acid, ephedrine and L-tyrosine through porcine pericardium

Ocena wpływu pH na *in vitro* przenikania kwasu salicylowego, efedryny i L-tyrozyny przez osierdzie wieprzowe

In our previous papers [1, 2] we studied permeation of peptide hormones through porcine pericardium. We showed that all the hormones are able to diffuse through the barrier however, the rates of diffusion measured as a percent permeated within 3 hrs varied from 16.4% (dalareline) up to 53–54% (tyreoliberine and prolactine). There was no correlation between the rate of penetration and the molecular weight of the hormone. The obtained pattern reminded the "u" curve. The smallest molecule (tyreoliberine) and the biggest (prolactine) showed the highest and almost the same rate of penetration. The lowest value obtained for dalareline was twice as low as for busereline having almost the same molecular weight. We were not able to explain such findings.

In the present paper we decided to study permeation of much simpler compounds having similar molecular weights under various pH conditions enabling diffusion of all the forms: free base or cation (ephedrine), free acid or anion (salicylic acid) and cation, inner salt or anion (L-tyrosine).

MATERIAL AND METHODS

The following materials were used: salicylic acid and sodium salicylate (Sigma-Aldrich, Germany), ephedrine and ephedrine hydrochloride Sigma-Aldrich, Germany), L-tyrosine (Sigma-Aldrich, Germany) and buffers (Sigma-Aldrich, Germany) in order to maintain the desired pH of the solution of L-tyrosine.

A standard Franz-type diffusion cell [3] was used (the volume of both donor and receptor chambers were 2 mililiters, the area of diffusion was 0.71 cm^2). Slices of frozen pericardium (coming from hearts of one-year-old pigs) were thawed in the same medium (around 50 ml) as used later for acceptor chamber, washed with the same medium and attached immediately between two halves of the cell. Acceptor chambers were previously filled with the same medium as donor ones without penetrating the chemical. Donor chambers were filled with 0.05% (salicylic acid and sodium salicylate) or 0.3% (ephedrine or ephedrine hydrochloride) solution of the studied compound in 0.9% of sodium chloride in water. In case of L-tyrosine appropriate buffers were used in order to maintain the desired pH value (2,2 ; 4 ; 5,6 ; 7 and 9,1), the concentration of amine acid was 0,1%. The system was then transferred into the mechanical shaker (UW type, Laboratory Equipment Manufacturer, Poland) and shaken at 120 min^{-1} and amplitude 3 cm. There was also a tiny teflon coated lead sphere

added into each acceptor chamber that enabled efficient stirring. The samples (the whole volume of acceptor chamber) were withdrawn with a syringe after 10, 20, 40, 60, 120, 180, 240 and 300 min (acceptor chamber was subsequently replenished with a suitable medium – 0.9% solution of sodium chloride in water or appropriate buffer). The temperature of the experiments was kept at 24° ± 2°C. The concentrations of penetrating chemicals were established using Specord UV-VIS spectrophotometer (Carl Zeiss, Jena) at the wavelength for maximal absorbance (salicylic acid – 303 nm, ephedrine – 257 nm and L-tyrosine – 275 nm) and then recalculated for the percentage of the initial dose that penetrated through the pericardium using calibration curves. Solutions of penetrating substances were chemically stable under applied conditions and they produced the same absorbance up to 24 hrs after preparation or removal from the acceptor chamber. Each experiment was repeated five times and the results were evaluated as the average. Additional experiments for every donor and acceptor medium (without salicylic acid, ephedrine nor L-tyrosine) were also carried out in order to check whether there is no diffusion of membrane components (as peptides are known to absorb UV at around 280 nm) that could affect absorbance of salicylic acid, ephedrine or L-tyrosine. No significant absorbance was measured.

RESULTS AND DISCUSSION

In this study we decided to use chemicals with similar molecular weights but different behaviour depending on pH values: ephedrine can be present at its cationic or free base form, salicylic acid – as an anion or free acid, L-tyrosine can be found as an anion, cation or inner salt. There are slight differences between penetration of ionized and non-ionized forms of salicylic acid and ephedrine – Figures 1 and 2.

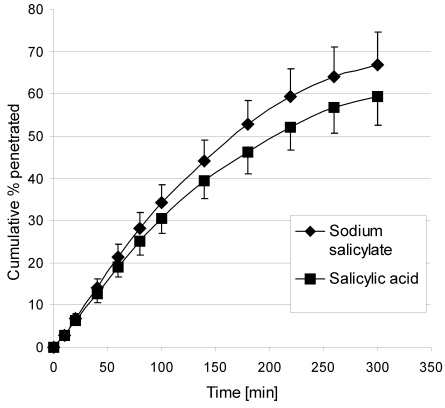

Fig. 1. Penetration of salicylic acid and its sodium salt through porcine pericardium

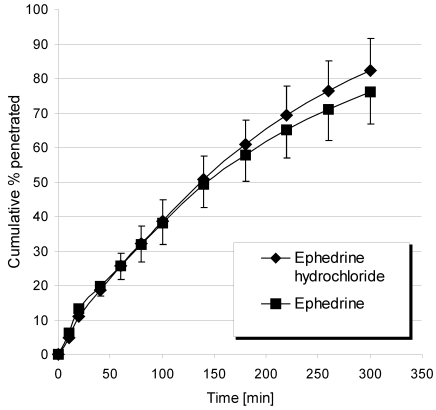

Fig. 2. Penetration of ephedrine and its hydrochloride through porcine pericardium

Sodium salicylate penetrates faster than salicylic acid (66.9% vs 59.4% up to 300 min). Even a smaller difference can be observed in the case of ephedrine – its hydrochloride penetrates faster than non ionized base (82.2% vs 76.0). Such findings are not in agreement with commonly accepted expectations that ionized forms penetrate more slowly than free acids or bases; however, the differences are really small in the case presented here. All the forms penetrate considerably faster (especially ephedrine) than all the hormones studied so far (16%–54%), to say nothing about iodide anion (around 5%) [1, 2]. These may be explained by relatively small molecular weights of salicylic acid and ephedrine in comparison to peptide hormones that are much heavier. Iodide ion shows strongly anionic character and even its tiny shape cannot help.

Interesting patterns can be observed in the case of L-tyrosine as we decided to establish pH for the experiments equals to pK_1 (carboxylic group), pK_2 (amine group), pI (isoelectric point) and – for the sake of a comparison– at pH equal to 4 and 7 as lying in between.

The fastest penetration can be observed at pH equaling to 2.2 (48.1%), then it decreases to 17.2% at the pH 5.6 and then increases to 35.2% at the pH 9.2 – Figures 3 and 4. The observed values cover almost the same range as we determined before [1, 2] for hormone peptides (16%–54%).

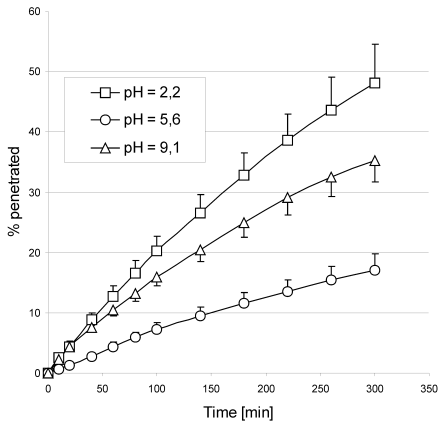

Fig. 3. Penetration of L-tyrosine at various pH through porcine pericardium
Patterns for pH 4 and 7 were omitted due to clarity reasons

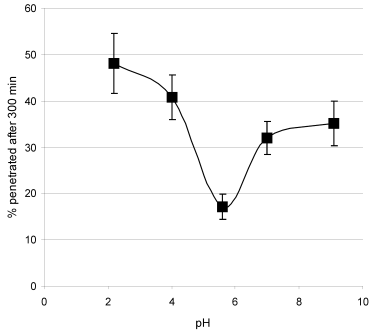

Fig. 4. Percent of initial amount of L-tyrosine penetrated after 300 min as a function of pH of the donor chamber

The cationic form shows the best diffusional properties, anionic form penetrates more slowly while inner salt is the worst penetrant. We must bear in mind that – according to Henderson-Haselbach equation – at pH = pK concentration of free base or acid equals concentration of its ionized form; therefore, we can expect (extrapolating the pattern from the Fig. 4) an increase of the penetration rate at pH lower than 2.2 and higher than 9.1 due to the increase of the concentrations of ionized forms of L-tyrosine. Nevertheless, such extreme pH conditions are likely to cause disruption of the pericardium membrane. On the other hand, pH values far from 7 are non physiological and are not likely to occur in living tissues.

The penetration pattern of peptides can be complicated by their amine acids composition and sequence that affects pH dependent behaviour of the molecule as the pK values of different amine acids are not the same. Also, the shape of the molecule caused by its secondary, tertiary and quaternary structure can play some role. Therefore, it seems that only experimental studies are able to show precisely how fast the penetration is and how the pH value of the donor medium affects penetration pattern. Of course, we are able to make some predictions according to the conclusions presented below.

CONCLUSIONS

From the data presented in this paper and the previous ones [1, 2] we can conclude that: 1. L-tyrosine penetrates the pericardium barrier at various rates depending on pH of the donor medium: the lowest rate is reached at isoelectric point with an increase of the penetration rate as a result of both a decrease and an increase of pH. The cationic forms diffuse faster than anionic ones. Ionized form of salicylic acid and ephedrine penetrates faster than the corresponding free acid and base; however, the differences are not significant. Cationic form of ephedrine penetrates faster than anionic form of salicylic acid. Such observations are in agreement with L-tyrosine behaviour observed in this study. 2. Strongly ionized anions such as iodide anions are expected to penetrate very slowly. 3. Peptides are expected to diffuse more slowly than smaller compounds as salicylic acid and ephedrine, but within the same range as in case of L-tyrosine. The observed penetration rate may mainly depend on pH of the donor medium – cationic forms should penetrate faster than anionic ones, while at isoelectric point penetration rate should reach its minimal value. Therefore, pH of the donor medium can be regarded as the most important factor determining peptides penetration.

REFERENCES

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SUMMARY

In the present paper we decided to study porcine pericardium permeation of simple compounds having similar molecular weights under various pH conditions enabling diffusion of all the forms: free base or cation (ephedrine), free acid or anion (salicylic acid) and cation, inner salt or anion (L-tyrosine). A standard Franz-type diffusion cell was used for all the experiments. Slices of frozen pericardium were thawed and attached immediately between two halves of the cell. Acceptor chambers were previously filled with the same medium as donor ones without penetrating the chemical. Donor chambers were filled with solution of studied chemicals at desired pH. The system was then transferred into the mechanical shaker, the samples were withdrawn and analysed using Specord UV-VIS spectrophotometer. We found that (contrary to commonly accepted predictions) ionised forms of salicylic acid and ephedrine penetrated faster than their nonionised forms; however, the differences were really small. All the forms penetrated considerably faster (especially ephedrine) than all the hormones studied so far (60%–80 % vs 16%–54%). In the case of L-tyrosine its cationic form showed the best diffusional properties, anionic form penetrated more slowly while inner salt was the worst penetrant. The observed values cover almost the same range (17%–48%) as we determined before for peptide hormones. The penetration pattern of peptides can be complicated by their amino acids composition and sequence that affects pH dependent behaviour of the molecule as the pK values of different amine acids are not the same. The molecule shape can also play some role. Therefore, it seems that only experimental studies are able to show precisely how fast the penetration is and how the pH value of the donor medium affects the penetration pattern. From all the studies we concluded that peptides are expected to diffuse more slowly than smaller compounds such as salicylic acid and ephedrine, but within the same range as L-tyrosine. The observed penetration rate may mainly depend on pH of the donor medium – cationic forms should penetrate faster than anionic ones, while at isoelectric point the penetration rate should reach its minimal value. Therefore, pH of the donor medium can be regarded as the most important factor.

STRESZCZENIE

W niniejszej pracy zbadano przenikanie przez osierdzie wieprzowe prostych związków o zbliżonej masie cząsteczkowej przy różnych wartościach pH umożliwiających dyfuzję wszystkich form: wolnej zasady lub kationu (efedryna), wolnego kwasu lub anionu (kwas salicylowy) oraz kationu, soli wewnętrznej lub anionu (L-tyrozyna). We wszystkich eksperymentach użyto standardowej celki dyfuzyjnej typu Franza. Kawałki zamrożonego osierdzia wieprzowego rozmrażano i natychmiast umieszczano pomiędzy połówkami celki. Komorę akceptorową uprzednio napełniano tym samym medium, co donorową, ale bez badanej substancji. Komorę donorową napełniano roz-

tworem badanego związku o żądanym pH. Taki zestaw przynoszono do wytrząsarki mechanicznej, a próbki pobierano i analizowano przy pomocy spektrofotometru Specord UV-VIS. Stwierdzono (wbrew powszechnie przyjętym oczekiwaniom), że zjonizowane formy kwasu salicylowego i efedryny przenikały szybciej niż formy niezjonizowane, chociaż różnice były bardzo małe. Wszystkie badane formy przenikały szybciej (szczególnie efedryna) niż wszystkie badane hormony (60% –80% wobec 16%–54%). W przypadku L-tyrozyny jej forma kationowa wykazała najlepsze właściwości dyfuzyjne, forma anionowa przenikała wolniej, a najwolniej sól wewnętrzna. Obserwowane wartości (17%–48%) zawierały się w niemal identycznym przedziale, jaki stwierdziliśmy poprzednio dla hormonów białkowych. Przenikanie peptydów może być skomplikowane przez ich skład aminokwasowy i sekwencję, które wpływają na zależne od pH zachowanie cząsteczki, ponieważ wartości pK różnych aminokwasów nie są identyczne. Kształt cząsteczki może również mieć pewien wpływ. Wydaje się zatem, że tylko badania doświadczalne mogą precyzyjnie pokazać, jak szybkie jest przenikanie i jak wpływa na nie wartość pH. Na podstawie wszystkich naszych badań możemy wnioskować, że peptydy powinny przenikać wolniej niż mniejsze związki, jak kwas salicylowy i efedryna, ale w podobnym zakresie jak L-tyrozyna. Obserwowane szybkości przenikania mogą głównie zależeć od wartości pH fazy donorowej – formy kationowe powinny przenikać szybciej niż anionowe, podczas gdy w punkcie izoelektrycznym szybkość przenikania powinna osiągać wartość minimalną. Zatem pH fazy donorowej można traktować jako najistotniejszy czynnik.