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Investigation of the effect of anaesthesia on nasal absorption of insulin in rats

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Abstract

This paper describes the investigation of the effect of a range of anaesthetics on the nasal absorption of insulin using the fall in blood glucose as an indication of insulin absorption. Insulin was administered as a solution in combination with the absorption enhancer sodium deoxycholate. Sagatal and Hypnorm anaesthetics were used as examples of parenterally administered anaesthetics and halothane was used as an example of an inhaled anaesthetic. One control group did not receive an anaesthetic. The greatest absorption was seen after anaesthesia with Sagatal and Hypnorm. The halothane group showed a smaller effect and the non-anaesthetised group showed little response. The study demonstrated that there is a difference in the absorption of nasally administered insulin in relation to the use of different anaesthetic agents. The differences in absorption are proposed to be due to an effect of the anaesthetic agents to a variable degree on the mucociliary clearance. © 1997 Elsevier Science B.V.

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1. Introduction

Many anaesthetic agents are available for use in animal experimental procedures ranging from short-acting inhalation agents such as halothane to longer acting intravenous agents such as sodium pentobarbital (Sagatal). The type of anaesthetic selected for a specific experiment depends on the type and duration of anaesthesia required. Hence for a study employing invasive surgical techniques Sagatal would be suitable whereas for the sedation of an animal while a non-invasive procedure was carried out Hypnorm, a shorter-acting anaesthetic, could be selected.

Numerous studies have been carried out in animal models on absorption of peptides from various mucosal sites including the nose, using different anaesthetics such as pentobarbital (Hirai et al., 1981; Illum et al., 1989; O'Hagan et al.,

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1990; Irie et al., 1992), urethane (Fix et al., 1986; Aungst and Rogers, 1989), ether (Aungst and Rogers, 1989) and ketamine (Daugherty et al., 1988; Schipper et al., 1990) during the procedure. Visor et al. (1986) investigated the nasal delivery of nicardipine in rats after anaesthesia with Sagatal and demonstrated the effect of the anaesthetic agent used in animals under surgery on the mucociliary clearance rate. The carotid artery was cannulated to allow blood sampling but no other surgery was carried out.

Several investigators have studied the effect of nasally administered drugs on nasal mucociliary clearance. Middleton et al. (1993) investigated the effect of amiloride and saline on the clearance in normal subjects and patients suffering from cystic fibrosis and Batts et al. (1989) investigated the effects of preservatives used in nasal preparations on the nasal clearance mechanism.

As far as we are aware no studies have been published on the investigation of the influence of different anaesthetics on the rate and degree of nasal absorption of drugs. Wolff et al. (1993) performed studies in which rhesus monkeys were anaesthetised with either ketamine alone or ketamine followed by Sagatal. The clearance of particles from the anterior and turbinate regions of the nasal cavity were measured but no drug was administered. The study showed that clearance was twice as fast in animals anaesthetised with ketamine alone indicating a longer residence time of a drug in the nasal cavity in animals anaesthetised with Sagatal. The reason for this effect was suggested to be due to ketamine being a dissociative anaesthetic that does not depress neural activity and therefore have no effect on the mucociliary clearance.

Illum et al. (1990) investigated the absorption of human growth hormone from the nasal cavity in sheep and while they found a significant increase in absorption using biodegradable starch microspheres the absorption of the formulation containing hGH alone was not as great as that previously noted by O'Hagan et al. (1990) when the same formulation was administered nasally to rats. Illum et al. (1990) and Illum (1996) suggested that this was due to the fact that in the latter study rats that had undergone an invasive surgical procedure under anaesthesia were used and in the study by Illum et al. (1990) conscious sheep were used. It was suggested that the difference in absorption could in part be due to the effect of the surgical procedure and anaesthesia on mucociliary clearance. Daugherty et al. (1988) also investigated the absorption of human growth hormone from the rat nasal cavity and found that there was significant absorption in the rat model anaesthetised with ketamine, acepromazine and zylazine, after administration of the growth hormone with an absorption enhancer. When the data was compared to absorption from the nasal cavity of nonanaesthetised rats it was found that there was less absorption in the non-anaesthetised model. The effect was suggested to be due to the lack of airflow through the nares of the unconscious, surgically treated model and the position of the animals on their backs with the possibility of pooling of the dose in the rear of the nasal cavity. This would affect the absorption of the drug.

The aim of the present study was to investigate the effect of various types of shorter or longer acting anaesthetics on the nasal absorption of the polypeptide insulin in a non-surgically treated rat model. Insulin was chosen as the model drug because its absorption into the blood stream is easily assessed by measuring the changes in blood glucose levels. The anaesthetic agents chosen for these studies were Sagatal and Hypnorm administered parenterally and halothane given by inhalation.

For comparison insulin was also dosed to non-anaesthetised rats. As a control animals were dosed with an insulin solution with no enhancer. This group was anaesthetised with halothane. In all experiments, but the control, insulin was administered in combination with sodium deoxycholate (SDC) in order to ensure some absorption of insulin in all rat models.

2. Methods

2.1. Materials

Sodium pentobarbital (Sagatal) and halothane were obtained from May and Baker, England.

Hypnorm was obtained from Janssen Pharmaceuticals. Porcine zinc insulin was provided by Novo Nordisk, Denmark. Sodium deoxycholate, sodium dihydrogen phosphate and disodium hydrogen phosphate were obtained from Sigma Chemicals.

2.2. Preparing the nasal formulation

A Zn-insulin stock solution was prepared by dissolving 69.4 mg of insulin in phosphate buffer at pH 4. The pH was adjusted to 7.4 by addition of sodium hydroxide and the solution was made up to 20 ml obtaining a final concentration of 80 IU/ml at pH of 7.4 The sodium deoxycholate stock solution was prepared by dissolving 1 g of sodium deoxycholate in 100 ml of phosphate buffer (pH 7.4) to give a final concentration of 1% w/v.

In order to obtain a working insulin-enhancer solution the two stock solutions were mixed in a ratio of 1:1 to give a final concentration of 40 IU/ml insulin and 0.5% w/v sodium deoxycholate.

2.3. Animal procedures

Groups of six male Wistar rats (Sutton Bonington, Nottingham University, UK) of about 250 g were fasted overnight but allowed water ad libitum. The groups were anaesthetised in the following way:

Group A was anaesthetised with an intraperitoneal (ip) injection of sodium pentabarbitone (Sagatal) at 60 mg/kg. The anaesthetic was given about 5 min before the first blood sample was taken. Group B was injected intramuscularly with 0.4 ml/kg of Hypnorm also approximately 5 min before the first blood sample was taken. Group C was anaesthetised with halothane at 2% in a 1:1 mixture of oxygen and nitrous oxide for 5 min. The baseline blood samples were taken before the animals were anaesthetised and the insulin dose was given immediately after the animals were anaesthetised. Group D was not anaesthetised in any way. Group E was anaesthetised with halothane in the same way as group C but dosed with 4 IU/kg insulin in phosphate buffer without sodium deoxycholate. This group was the control

No top-up anaesthesia was given during the course of the study and no surgical procedures were carried out.

The animals were dosed nasally into one nostril using a 100-μl Hamilton syringe with an attached length of polyethylene tubing (I.D. 0.4 mm, O.D. 0.8 mm). For delivery the tubing was inserted about 0.5 cm into the nostril (marked on the tube). Each animal was dosed with about 20 μl of the insulin enhancer solution to give a dose of 4 IU/kg insulin per animal.

The blood samples were taken at -10, -5 before dosing the animal and at 5, 10, 20, 30, 45, 60, 90, 120, 180, 240 min after dosing from the caudal vein using a needle with the hub broken off. Ten drops of blood were dropped into Sarstedt tubes containing sodium fluoride and heparin. The blood samples were tested for blood glucose levels using a Yellow Springs 23AM Glucose analyser (Yellow Springs Instruments, Ohio, USA).

3. Analysis of data

The area under the percent blood glucose against time plots (AUC) were calculated for the various groups of animals using the trapezoidal method. This allowed a direct comparison of the effect of anaesthesia on total fall in blood glucose between the groups. The smaller the AUC value the larger the fall in blood glucose concentration. Analysis of variance (ANOVA) was carried out between these values and unpaired t-tests were carried out to determine which values were significantly different. All statistical tests were evaluated at the 5% significance level.

ANOVA and t-tests were also performed on the minimum blood glucose levels obtained (expressed as percentage of basal level, C_{min}).

4. Results

Fig. 1 shows the effect of the various anaesthetics on the absorption of insulin in combination

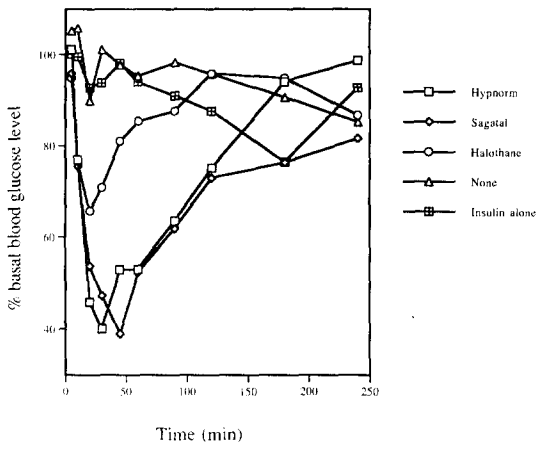

Fig. 1. The influence of anaesthetic agents on the nasal absorption of insulin in rats expressed as the change in blood glucose levels compared with a non-anaesthetised control group and insulin administered without any sodium deoxycholate.

with SDC, from the nasal cavity of the rat expressed as the percentage change in blood glucose levels. The control shows no significant fall in percent blood glucose. As can be seen, the animals anaesthetised with Sagatal and Hypnorm show the greatest falls (about 40% of the basal value) in blood glucose for both anaesthetics at about 30 min after administration of the insulin dose. Those animals anaesthetised with halothane show a fall to about 65% whereas the non-anaesthetised rats show no significant fall in blood glucose as compared to the control. Table 1 gives the AUC values obtained, the minimum percentage concentration of blood glucose as compared to basal levels observed throughout the study (C_{min}) and the time at which the minimum blood concentration occurred (t_{min}) for the various groups of animals.

The AUC values obtained for Sagatal and Hypnorm are significantly different to those obtained for the halothane group and for the non-anaesthetised group. There is no significant difference between the AUCs obtained for the halothane and non-anaesthetised groups. ANOVA further shows that there is a significant difference between all C_{min} and t_{min} values except for the Sagatal and hypnorm groups.

5. Discussion

The present study investigated the effect of anaesthetic agents on the nasal absorption of insulin from the nasal cavity of rats. Three different anaesthetics were employed: (i) Sagatal, to represent a commonly used agent in nasal absorption experiments in the rat model. Sagatal decreases blood pressure by peripheral vasodilation. The consequent blood pooling causes a decreased venous return leading to a decreased cardiac output (Vickers et al., 1991) which again causes a decreased blood supply to the peripheral blood vessels. (ii) Hypnorm, to represent an anaesthetic agent that, while sedating and paralysing the animal, does not render it unconscious. Fentanyl, one of the main agents in Hypnorm, is a synthetic morphine-like drug normally used as an adjunct to anaesthesia. Although the animal is paralysed with loss of feel it is still conscious. Cells react to direct action of opioids by hyperpolarisation, inhibition of cell firing and pre-synaptic inhibition of transmitter release. Fentanyl has the normal opioid responses of analgesia and respiratory depression. There is increase in vascular tone causing increase in blood flow (Rang and Dale, 1987).

Table 1
Effect of different anaesthetic agents on blood glucose level after nasal administration of insulin (4 IU/kg) in rat

Anaesthetic	Mean AUC (± S.D.)	C_{min} (% ± S.D.)	t_{min} (min)
Sagatal (60 mg/kg)	15 275 (± 1877)	47.2 (± 19)	30
Hypnorm (0.4 ml/kg)	17 669 (± 2591)	40 (± 10)	30
Halothane (2% in 1:1 O_2/NO for 5 min)	20 708 (± 1514)	65.7 (± 16)	20
None	22 008 (± 1812)	95.2 (± 16)	60
Control	20 240 (± 1499)	76.3 (± 8)	180

Both Sagatal and Hypnorm are given parenterally by intravenous, intraperitoneal, subcutaneous or intramuscular injections. (iii) Halothane was selected to represent an agent given by inhalation. Halothane is short acting and considered to influence to a lesser extent the normal physiological processes in the animal. Halothane causes a decrease in blood pressure by a decrease in myocardial contractility and cardiac output (Vickers et al., 1991). Halothane does cause an increase in the frequency of breathing that may have an effect on the absorption of nasally administered drugs.

The lowest blood glucose levels obtained after nasal administration of insulin were seen in the groups of animals anaesthetised with Sagatal and Hypnorm. This was expected as both these agents are given parenterally and have a long duration of action (recovery starts after about 30 min). During the time the rats were anaesthetised they were immobilised so the insulin dose would be expected to reside in the nasal cavity longer than if the animals were mobile.

Olanoff et al. (1987) investigated the effect of nasal blood flow on the absorption of desmopressin by using histamine to increase the nasal blood flow. They found that when administered in conjunction with histamine, desmopressin caused a greater diuretic effect compared to when it was administered alone. This could be due to the effect of the histamine on the permeability of the nasal mucosa but the authors suggested that in the low concentrations administered the effect on the nasal mucosa would be negligible and that the increase in absorption seen was due to the increase in blood flow creating a greater concentration gradient across the mucosa. It was expected that animals anaesthetised with Hypnorm would show a larger absorption of insulin relative to animals anaesthetised with Sagatal because of the increase in blood flow caused by the former agent (Rang and Dale, 1987) and the fact that there is a reduced blood flow to the peripheral blood vessels after anaesthesia with Sagatal (Vickers et al., 1991). however, there was no significant difference in the AUC values of the C_{min} values obtained for these two groups. This indicates that the amount of blood flow to the nose is not the critical factor in determining extent of absorption from the nasal cavity in this animal model. The fact that the rats are immobilised and the likely effect of the anaesthesia on mucociliary clearance rate by both agents probably plays a more significant role in determining the extent of absorption.

The rats anaesthetised with halothane recovered in 3–5 min which was reflected in the extent of absorption of insulin in this group. There was a fall in blood glucose but the AUC values were not significantly less than those found in the non-anaesthetised group. The AUC values were significantly greater than those in the Sagatal and Hypnorm groups. This indicates a significantly lower absorption of insulin when the animals are anaesthetised with halothane.

The C_{min} values however show that there was a significant difference in the minimum blood concentration between the halothane group and the non-anaesthetised group. After anaesthesia with halothane the rats were immobilised sufficiently long to ensure accurate dosing but the mucociliary clearance were most likely not influenced to a significant degree by this anaesthetic agent. These results are in agreement with those of Illum et al. (1990) and Daugherty et al. (1988) both of whom found that absorption of peptides was greater in animals that had been anaesthetised with a long acting parenteral anaesthetic.

Duerbo et al. (1989) and Merkus et al. (1991) investigated the effects of interspecies variation on the nasal absorption of insulin. The former authors found that without an absorption enhancer rabbits showed a greater bioavailability relative to rats whereas after addition of an absorption enhancer dimethyl-β-cyclodextrin the reverse was true. It was also suggested in this report that the differing experimental conditions may have contributed to this result. Merkus et al. (1991) investigated the difference in absorption of insulin after nasal administration to rats, rabbits and humans and found large variation in the effect with nearly 100% absorption in rats, 2–10% in rabbits and no detected absorption in humans.

Baldwin et al. (1990) and Illum et al. (1990) reported the interspecies variation after administration of human growth hormone (hGH). Baldwin examined absorption after administration to three different species of animals (rats, rabbits and sheep) under different experimental conditions and reported a variation in absorption. Illum et al. (1990) reported a difference in absorption between the sheep model and the rat model. In both the above cases the rat model underwent a surgical procedure under anaesthesia which probably contributed to the difference in absorption.

Very few studies have been reported on the effect of different experimental conditions in the same animal species on absorption of drugs nasally. Daugherty et al. (1988) showed that after nasal administration of hGH to rats submitted to a surgical procedure under anaesthesia and to rats under no surgical treatment a large difference in absorption was seen. This is in agreement with the present study. It should be noted that the difference could also in part be due to the surgical procedure and problems in dosing conscious rats. Auger et al. (1990) investigated the effect of anaesthetic gases on the cilia beat frequency and found it to be unaffected but there was a fall in the number of ciliated cells found in the trachea after anaesthesia. This further reinforces the findings of the present study that anaesthesia by inhalation anaesthesia does not significantly inhibit the mucociliary clearance mechanisms.

Tacheuchi et al. (1990) investigated the effect of parenterally administered atropine on the mucociliary clearance rates. They found that the clearance was slowed which was attributed to the reduction in the mucus volume rather than an effect on the ciliary beat frequency. In the present study it is not possible to suggest whether the effect seen is also due to effects on other than the immobilisation of the animals and the probable effect on mucociliary clearance. Fisher et al. (1992) reported that the position of the rat during the experiment may affect the absorption of nasally administered drugs by changes in the drainage and ciliary functions in different positions. This was eliminated in the present study by placing the animals on their fronts immediately post-dosing. It should be noted that the non-anaesthetised group were at time difficult to dose and that the rats frequently 'sneezed'. Hence, there is a possibility that this group of rats were not always fully dosed.

This study demonstrated that there is a difference in the absorption of nasally administered insulin when animals are anaesthetised with different anaesthetic agents or are fully conscious during the whole experiment. The differences seen are proposed to be due to an effect on the mucociliary clearance mechanism rather than a possible pharmacological effect of the anaesthetic agent on blood flow, etc. Care should be taken when extrapolating results obtained in anaesthetised animal models to man.

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References

Auger, C., van Bommel, T., Phadana-anek, S., Reich, A., Butler, J., Stahl, H. and Deimer, T., Behaviour of relative humidity and temperature in the inspiratory part of a Drager circle system and their influence on the functioning of the ciliary epithelieum. *Anasth. Intensivther. Notfallmed.*, 25 (1990) 107–111.

Aungst, B.J. and Rogers, N.J., Comparison of the effects of various transmucosal absorption promoters on the effect of buccal insulin delivery. *Int. J. Pharm.*, 53 (1989) 227–235.

Baldwin, P.A., Corine, K.K, Grimm, C.J. and Longenecker, J.P., The effect of sodium tauro 24,25-dihydrofusidate on the nasal absorption of human growth factor in three animal models. *Pharm. Res.*, 7 (1990) 547–552.

Batts, A.H., Martin, G.P. and Bond, S.W., The effect of some preservatives used in nasal preparations on mucociliary clearance. *J. Pharm. Pharmacol.*, 41 (1989) 156–159.

Daugherty, A.L., Liggit, H.D., MCCaobe, J.G., Moore, J.A. and Patton, J.S., Absorption of recombinant-methionyl-human growth hormone from rat nasal mucosa. *Int. J. Pharm.*, 45 (1988) 197–206.

Duerbo, M.J., Hermens, W.A.J.J., Romeyn, S.G., Verhoef, J.C. and Merkus, F.W.H.M., Absorption enhancement of intranasally administered insulin by sodium taurodihydrofusidate (STDHF) in rabbits and rats. *Pharm. Res.*, 6 (1989) 854–856.

Fisher, A.N., Illum, L, Davis, S.S. and Schacht, E.H., Diiodotyrosine labelled dextrans as molecular size markers of nasal absorption in the rat. *J. Pharm. Pharmacol.*, 44 (1992) 550–554.

Fix, J.A., Engle, K., Porter, P.A., Leppet, P.S., Selk, S.J., Gardner, C.R. and Alexander, J., Acylcarnitines: drug absorption enhancing agents in the gastrointestinal tract. *Am. J. Physiol.*, 251 (1986) G332–G340.

Hirai, S., Yashiki, T., Matsuzawa, T. and Mima, H., Absorption of drugs from the nasal mucosa of rat. *Int. J. Pharm.*, 7 (1981) 317–326.

Illum, L., Nasal delivery. The use of animal models to predict performance in man. *J. Drug Target.*, 3 (1996) 427–442.

Illum, L., Farraj, N.F., Critchley, H., Johansen, B.R. and Davis, S.S., Enhanced absorption of insulin in rats using lysophosphatidylcholine. *Int. J. Pharm.*, 57 (1989) 49–54.

Illum, L., Farraj, N.F., Davis, S.S., Johansen, B.R. and O'Hagan, D.T., Investigation of the nasal absorption of biosynthetic human growth hormone in sheep: use of a bioadhesive microsphere delivery system. *Int. J. Pharm.*, 63 (1990) 207–211.

Irie, T., Wakamatsu, K., Arima, H., Aritomi, H. and Uekama, K., Enhancing effects of cyclodextrins on nasal absorption of insulin in rats. *Int. J. Pharm.*, 84 (1992) 129–139.

Merkus, F.W.H.M., Verhoef, J.C., Romeijn, S.G. and Schipper, N.G.M., Interspecies differences in the nasal absorption of insulin (letter). *Pharm. Res.*, 8 (1991) 1343.

Middleton, P.G., Geddes, D.M. and Alton, E.W.E.W., Effect of amiloride and saline on nasal mucociliary clearance and potential difference in cystic fibrosis and normal subjects. *Thorax*, 48 (1993) 812–816.

O'Hagan, D.T., Critchley, H., Farraj, N.F., Fisher, A.N., Johansen, B.R., Davis, S.S. and Illum, L., Nasal absorption enhancers for the biosynthetic human growth hormone in rats. *Pharm. Res.*, 7 (1990) 772–776.

Olanoff, L.S., Titus, C.R., Shea, M.S., Gibson, R.E. and Brooks, C.D., Effect of intranasal histamine on nasal blood flow and the antidiuretic activity of desmopressin. *J. Clin. Invest.*, 80 (1987) 890–895.

Rang, H.P. and Dale, M.M., *Pharmacology*, Churchill Livingstone, London, 1987, pp. 471–485.

Schipper, N.G.M., Hermens, W.A.J., Romeyn, S.G., Verhoef, J. and Merkus, E.W.H.M., Nasal absorption of 17-beta-estradiol and progesterone from a dimethyl-cyclodextrin inclusion formulation in rats. *Int. J. Pharm.*, 64 (1990) 61–66.

Tacheuchi, T., Suzumura, E., Majima, Y. and Sakakura, Y., Effect of atropine on nasal mucociliary clearance. *Acta Otolaryngol*, 110 (1990) 120–123.

Vickers, M.D., Morgan, M. and Spencer, P.S.J., *Drugs in anaesthetic practice*. Butterworth and Heineman, London. 1991.

Visor, G.C., Bajka, E. and Benjamin, E., Intranasal delivery of nicardipine in the rat. *J. Pharm. Sci.*, 75 (1986) 44–46.

Wolff, R.K., Allen, D.L., Hughes, B.L., Osier, M. and Dorato, M.A., Nasal clearance in rhesus monkeys. *J. Aerosol Med.*, 6 (1993) 111–119.