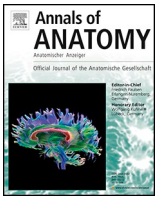
Annals of Anatomy 254 (2024) 152240

Contents lists available at ScienceDirect

Annals of Anatomy

journal homepage: www.elsevier.com/locate/aanat



Postmortem sympathomimetic iris excitability

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ARTICLE INFO

Keywords:
Supravital reactions
Postmortem chemical iris excitability
Compound method
Postmortem interval

ABSTRACT

Background:: A reliable estimation of time since death can be important for the law enforcement authorities. The compound method encompassing supravital reactions such as the chemical excitability of the iris can be used to further narrow intervals estimated by temperature-based methods. Postmortem iris excitability was mostly assessed by parasympatholytic or parasympathomimetic substances. Little is known regarding sympathomimetic agents. The present study aims to describe the postmortem iris excitability using the sympathomimetic drug phenylephrine.

Methods:: Cadavers were included after body donors gave written informed consent during lifetime. Exclusion criteria were known eye disease, or a postmortem interval exceeding 26 hours. A pupillometer with a minimum measurement range of 0.5 mm was used to determine the horizontal pupil diameter before and 20 minutes after the application of phenylephrine. Increase in pupil diameter was labeled as positive reaction, unchanged pupil diameter was labeled as negative reaction, and decrease in pupil diameter was labeled as paradox reaction.

Results:: 30 eyes from 16 cadavers (median age = 80.0; 9 males, 7 females) were examined. Initial pupil size was in median 3.5 mm (interquartile range [IQR]: 3.0–4.5 mm) and progressed to 4.0 mm (IQR: 3.5–5.0 mm) 20 minutes after drug instillation. The achieved pupil diameter difference comprised in median 0.5 mm (IQR: 0.0–1.0 mm). A positive reaction was observed in 21 cases. Negative reactions were observed in 5 cases and paradox reactions in 4 cases. Overall, there was a statistically significant difference in diameter between the initial and the reactive pupil ($P = 0.0002$).

Conclusion:: Although relatively rarely used, sympathomimetic drugs seem to be eligible for chemical postmortem iris excitability. Currently, assessment of postmortem iris excitability usually only involves parasympatholytic and parasympathomimetic agents. The findings of the present study give a hint that the application of a third agent with a sympathomimetic mechanism of action could provide additional information. Further studies assessing such a triple approach in the compound method in comparison with the current gold standard for estimation of time since death are mandatory to ensure reliable results.

1. Introduction

The central task of legal medicine is the provision of expert medical opinion for the law enforcement authorities and the respective jurisdiction (Beran, 2013). By that, the estimation of time since death in a particular case can be important (Madea, 2016) and can influence the investigative inquiry (Henssge et al., 2000b). Thus, the reliability of the estimation of the time since death is mandatory (Madea, 2016). The gold standard for the estimation of time since death is considered to be the temperature-based nomogram method (Madea, 2016). Furthermore, the temperature-based method can be combined with other methods such as the compound method by the deployment of conditional probability distributions (Biermann and Potente, 2011; Potente and Biermann, 2021). The compound method itself already combines the rectal temperature-based nomogram (Henssge et al., 2000b) and non-temperature-based methods (Henssge et al., 2000a). The latter comprise classical postmortem findings such as rigor mortis and lividity but also among others the chemical excitability of the iris (Henssge

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https://doi.org/10.1016/j.aanat.2024.152240
Received 26 January 2024; Received in revised form 20 February 2024; Accepted 5 March 2024
Available online 7 March 2024

et al., 2000a). By that, the eye can become an organ of versatile interest in forensic medicine (Ang et al., 2021). The postmortem excitability of the iris belongs to the complex of supravital reactions and is based on the prolonged survival of certain cell types after the organism's death (Ang et al., 2021). As a result, there is a time limit regarding postmortem iris excitability. For example, it has been demonstrated that acetylcholine, as parasympathomimetic agent, and tropicamide, as parasympatholytic, can initiate pupil reactions in a postmortem interval of up to 46 and 30 h, respectively (Klein and Klein, 1960). The anatomic correlate of these supravital reactions is the iris that hosts both, the sympathetic musculus dilatator pupillae and the parasympathetic musculus sphincter pupillae (Neuhuber and Schrodl, 2011). Although the iris excitability does not meet the criterion of a reliable postmortem interval estimation (Koehler et al., 2018; Orrico et al., 2008), enhancing the knowledge on the postmortem iris excitability potentially yields new starting points for advances in the compound method. As most studies so far employed parasympatholytic or parasympathomimetic substances, little is known on the postmortem chemical iris excitability induced by sympathomimetic agents. Nevertheless, older studies reporting the use of sympathomimetics such as (nor-)adrenaline or phenylephrine exist, but they are hardly accessible, with methods and results often insufficiently described, directly evidencing the need for additional investigations (Bardzik, 1966; Jaafar and Nokes, 1994; Klein and Klein, 1960, 1978). Therefore, the aim of the present pilot study was to investigate the effect of the less common sympathomimetic agent phenylephrine on postmortem iris excitability.

2. Material and methods

2.1. Study design

The present pilot study based on cadaveric tissue was designed to investigate the postmortem iris excitability by administering the sympathomimetic agent phenylephrine. Information on the postmortem interval and preexisting diseases were collected from the death certificate of each body donor (Table 1). Inclusion criterion for the cadavers used was written informed consent to the scientific postmortem use of the body of the body donor. Exclusion criteria were known eye disease or a postmortem interval $>$ 26 h, as described previously (Larpkrajang et al., 2016; Orrico et al., 2008). The cadavers were stored at 13°C and prepared as displayed in Fig. 1 at the Saarland University's Institute for Anatomy and Cell Biology. Horizontal pupil diameter was determined using a pupillometer with a minimum measurement range of 0.5 mm according to the manufacturer's information before and 20 minutes after drug application (SO1, Schneider Ophtoshop, Friedberg, Germany). Based on a previous study that indicated a minimum time window of 5–10 min for mydriatics (Bardzik, 1966; Jaafar and Nokes, 1994), the latter measurement time of 20 min after drug instillation was set empirically. Two drops of phenylephrine were administered to each of the 30 eyes (Neosynephrin-POS 10%, 100 mg/ml, Ursapharm, Saarbrücken, Germany). Increase in pupil diameter was labeled as positive reaction, unchanged pupil diameter was labeled as negative reaction, and decrease in pupil diameter was labeled as paradox reaction.

2.2. Statistical analysis

Non-continuous variables were described by absolute and relative frequencies. Continuous variables were described by median and interquartile range (IQR). The initial and treated pupil diameters were compared using the non-parametric Wilcoxon-signed-rank-test for paired samples. Statistical analysis was performed using the GraphPad Prism Software Package (Version 10.0.3, GraphPad Software, Inc). A significance level of $\alpha = 0.05$ was defined, thus *P*-values (labeled as "*P*") below 0.05 were rated as statistically significant.

Table 1
Baseline characteristics of the assessed cadavers.

Donor Number	Age	Sex	Postmortem Interval	Cause of death according to Death certificate
1	96	Female	21.8	Multi-organ failure, chronic heart disease, arterial hypertension, dementia
2	79	Male	19.2	Multi-organ failure, sepsis, pneumonia, dementia with dysphagia
3	78	Female	22.5	Cardiovascular failure, exsiccosis with kidney disease, obstructive ileus
4	87	Male	9.0	Asphyxia, lung carcinoma, fibrotic lung disease
5	76	Male	18.0	Sudden cardiac death, arterial hypertension
6	80	Male	9.5	Lung carcinoma, chronic heart disease, arterial hypertension
7	88	Female	13.3	Multi-organ failure, arterial hypertension, Alzheimer's dementia
8	99	Male	3.2	Multi-organ failure, Parkinson's disease, diabetes mellitus
9	73	Female	15.4	Extensive stroke, advanced bronchial carcinoma
10	89	Female	16.9	Klatskin tumor, sepsis, renal failure, Meniere's disease
11	94	Female	15.6	Respiratory insufficiency, pneumonia, acute renal failure, anemia, chronic heart disease, arterial hypertension
12	64	Male	16.6	Encephalitis
13	68	Male	20.8	Cardiac failure, chronic heart disease
14	82	Female	9.1	Urinary bladder carcinoma, diabetes mellitus, multiple petechial hemorrhages
15	80	Male	10.0	Cancer of unknown primary, diabetes mellitus, arterial hypertension
16	79	Male	13.3	Metastatic anal carcinoma, spondylodiscitis L3-S1, septic shock, renal failure

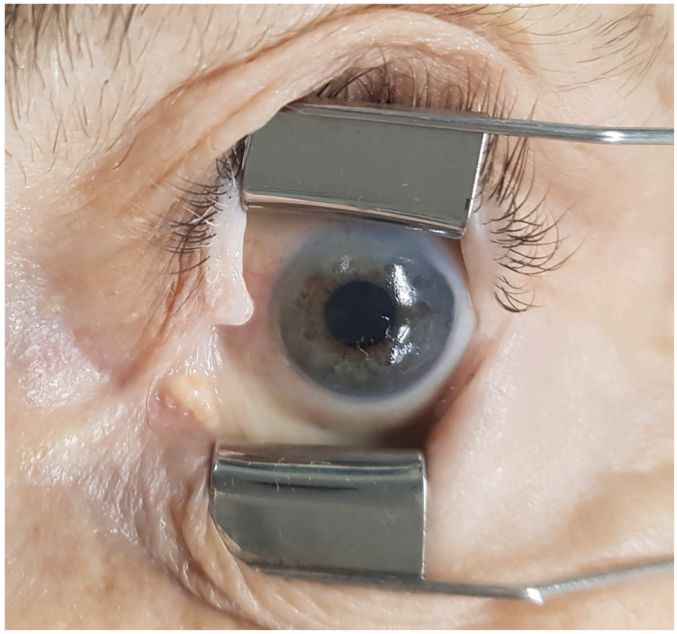

Fig. 1. Cadaver eye prepared for drug administration.

3. Results

Overall, phenylephrine was applied to a total of 30 eyes from 16 different cadavers. Both eyes were investigated in a total of 14 cadavers, whereas only one eye was examined in the remaining two cadavers. Of all the cadavers examined, 9 were male (56.25%) and 7 were female (43.75%). The median age was 80.0 years (IQR: 76.5—88.8 years). Based on the individual body donors, the median postmortem interval until drug application was 15.5 hours (IQR: 9.6—18.9 hours) (Table 1 and Table 2).

Initial pupil size was 3.5 mm (IQR: 3.0–4.5 mm) and progressed to 4.0 mm (IQR: 3.5–5.0 mm) 20 minutes after drug application. The achieved pupil diameter difference comprised 0.5 mm (IQR: 0.0–1.0 mm). In 21 cases (70.0%), a positive reaction was observed. Pupil diameter remained unchanged in 5 cases (16.7%), whereas inverse "paradox" reactions were described in 4 cases (13.3%). Wilcoxon-signed-rank-test indicated a statistically significant difference in diameter between the initial and the reactive pupil ($P = 0.0002$). The different measurements per eye are displayed in Table 2.

4. Discussion

Estimation of time since death is an important task for legal medicine experts to support law enforcement authorities and jurisdiction (Beran, 2013). In addition to the gold standard, the temperature-based nomogram method, other methods such as the compound method can support the reliable estimation of time since death (Henssge et al., 2000a, 2000b; Madea, 2016). The compound method among others also considers the postmortem pharmacological iris excitability. Based on sympathetic and parasympathetic innervation of antagonistic iris musculature, supravital reactions of the iris can either be of mydriatic or miotic nature. Different classes of pharmacological agents are conceivable and include sympathomimetics (e.g., phenylephrine, adrenaline) and parasympatholytics (e.g., tropicamide, atropine) to dilate, and parasympathomimetics (e.g., acetylcholine, pilocarpine) and sympatholytics to constrict the pupil. As the chemical excitability is time-sensitive, such an approach is limited to the early postmortem interval, as exemplified by a study that observed last constrictive effects of pilocarpine after 15 hours (Larpkrajang et al., 2016). However, even after longer postmortem intervals pupillomotion can be pharmacologically induced in some instances (Koehler et al., 2018). Studies so far, such as the study by Orrico and colleagues, investigated both atropine and pilocarpine using two different drug application methods (Orrico et al., 2008). In that study only 18.8% of 309 eyes displayed a positive reaction to atropine, and only 16.2% of the other 309 eyes to pilocarpine (Orrico et al., 2008). The positive reactions were observed in 21%, 17%, and 20% (atropine) as well as 23%, 12%, and 15% (pilocarpine) within the time frame of 0–6, 6–10, and 10–26 h, respectively. To our knowledge, no such upper time limits have been described for phenylephrine until now. In our study, positive reactions were observed until a postmortem interval of 22.5 h. We observed paradox reactions in 13.3% of all cases. These might be due to spontaneous pupil dynamics, which we did not assess in this explorative pilot study. It was interesting to notice, that these reactions never occurred in both eyes of the same patient (see Table 2). However, in three of the four cases, the neighbor eye displayed a negative reaction, whereas only once a positive reaction was noticed. This supports the previous observation that spontaneous postmortem pupil diameter dynamics are highly individual (Fleischer et al., 2017). As aforementioned, the use of sympathomimetics to study postmortem iris excitability was seldom reported, and if it was, then often insufficiently (Bardzik, 1966; Jaafar and Nokes, 1994; Klein and Klein, 1960, 1978). In this context, essentially adrenaline and noradrenaline were investigated. Phenylephrine, a synthetic sympathomimetic, was only addressed by Klein and Klein (Klein and Klein, 1978). They studied 63 cases of subconjunctival instillation and 75 cases of anterior chamber injection with 10% phenylephrine. To the best of our knowledge, changes in pupil diameter were not measured, but only labelled as positive or negative. Apparently, the labelling as negative also comprised paradox reactions, that were only observed when the mydriatic was injected in the anterior chamber. Pupilloconstriction was never observed after subconjunctival instillation. Within a postmortem interval of 20 hours, 27 eyes displayed a positive and 5 a negative reaction. Beyond 20 hours, most reactions were negative (Klein

Table 2
Pharmacological iris excitability per donor and per eye.

Donor Number	Postmortem Interval	Initial Pupil Diameter	Treated Pupil Diameter	Pupil Diameter Difference	Cadaver Sex	Eye Side
1	21.8	4.5	5.0	+0.5	Female	Right
2	19.2	4.5	5.0	+0.5	Male	Right
2	19.2	3.5	4.0	+0.5	Male	Left
3	22.5	2.5	4.0	+1.5	Female	Right
3	22.5	3.0	3.5	+0.5	Female	Left
4	9.0	4.5	5.0	+0.5	Male	Right
4	9.0	2.5	4.0	+1.5	Male	Left
5	18.0	4.0	4.0	0.0	Male	Right
5	18.0	3.0	2.5	−0.5	Male	Left
6	9.5	3.0	4.0	+1.0	Male	Right
6	9.5	4.5	4.0	−0.5	Male	Left
7	13.3	3.0	3.5	+0.5	Female	Right
7	13.3	3.5	4.0	+0.5	Female	Left
8	3.2	3.0	3.5	+0.5	Male	Right
8	3.2	2.5	3.0	+0.5	Male	Left
9	15.4	3.5	3.5	0.0	Female	Right
9	15.4	3.5	3.0	−0.5	Female	Left
10	16.9	4.5	6.0	+1.5	Female	Right
10	16.9	4.0	5.5	+1.5	Female	Left
11	15.6	3.0	3.5	+0.5	Female	Right
11	15.6	3.0	4.0	+1.0	Female	Left
12	16.6	5.5	5.0	−0.5	Male	Right
12	16.6	5.5	5.5	0.0	Male	Left
13	20.8	3.0	4.0	+1.0	Male	Left
14	9.1	4.0	4.0	0.0	Female	Right
14	9.1	4.5	5.0	+0.5	Female	Left
15	10.0	4.5	5.5	+1.0	Male	Right
15	10.0	4.5	4.5	0.0	Male	Left
16	13.3	3.5	4.5	+1.0	Male	Right
16	13.3	4.0	4.5	+0.5	Male	Left

and Klein, 1978). The rarity of such data, the putative absence of quantitative pupil diameter change analysis, and the yet ongoing developments in postmortem interval estimation in legal medicine highlight the critical interest of our study, further evidencing the potential of postmortem sympathomimetic iris excitability. In this context, phenylephrine becomes an additional option next to (nor-)adrenaline that was adequately reported by Klein and Klein (Klein and Klein, 1978). The interest in several sympathomimetic agents might lie in different drug potencies, that might enable further interval grading. Indeed, considering our results and those from Klein and Klein, one should attribute a lower potency to phenylephrine compared to (nor-)adrenaline (Klein and Klein, 1978). However, further research is needed to unravel and evidence these nuances and their use for postmortem interval estimation.

A few limitations have to be mentioned at this point. The number of investigated eyes in this pilot study was limited. Hence, future studies with a sufficiently high number of cases on such agents would be helpful to also control for potential statistical β-fault. The present study investigated both eyes of most specimens, as done previously (Orrico et al., 2008). To prevent influences from the contralateral eye some studies only addressed one eye per cadaver (Koehler et al., 2018; Larpkrajang et al., 2016). However, since the nervous system is not functional postmortem and the observed mydriatic or miotic reaction should remain a local supravital muscle reaction, such interactions seem questionable. Koehler *et al.* added thereto that observed "consensual" reactions in the untreated neighbor eye rather corresponded to spontaneous than to systematic alterations due to contralateral drug instillation (Koehler et al., 2018). Additionally, based on the previous study from Klein and Klein that indicated a high number of paradox reactions after anterior chamber injection (Klein and Klein, 1978), we have chosen the non-invasive method of conjunctival drug instillation. However, more methodological studies than the one cited are needed to compare the application of phenylephrine into the anterior eye chamber and / or the conjunctival sac. Furthermore, the present study did not comprise negative controls. Also no longitudinal measurements over time have been conducted, even though some studies reported aforementioned spontaneous postmortem changes of the pupil size (Ang et al., 2021). Future studies could also assess different methods to determine pupil diameter and analyze the inter- and intra-observer reliability of the different methods. Indeed, the pupillometer employed, even though standardized, is another limitation of our study, displaying a rather large minimum measurement range of 0.5 mm.

5. Conclusion

Even though controls are missing, our study provides further evidence that phenylephrine can be used as mydriatic postmortem agent in form of eye drop instillation. Thereby, the sympathomimetic mechanism of action is supposed to be more and more susceptible to complement the currently more frequently used parasympatholytic and parasympathomimetic drugs. Thus, the findings of the present pilot study can be seen as a hint that a triple combination to assess this supravital phenomenon could be feasible. Further studies addressing the corresponding benefit of sympathomimetics in this context are needed. Subsequently comparative studies among such triple combinations and both the current gold standard for the estimation of time since death and the conventional compound method are mandatory to assess the diagnostic value of such an approach.

Ethical statements

The cadavers used were those of body donors who gave written informed consent during lifetime for the postmortem use of their body for research and education. They were obtained from the body donation programme of the Saarland University. The local ethics committee approved the present study (vote number 236/22; "Ständige Ethikkommission der Ärztekammer des Saarlandes"). The research was performed in accordance with the guidelines from the Declaration of Helsinki.

Funding

This research received no external funding.

CRediT authorship contribution statement

Thomas Tschernig: Conceptualization. **Cristina Martin Lesan:** Methodology. **Berthold Seitz:** Writing – review & editing, Supervision. **Reem Alrefai:** Methodology, Investigation. **Colya N. Englisch:** Writing – original draft, Investigation.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Acknowledgements

The authors' gratitude goes to Christina Turner who spell-checked the language. The authors also wish to sincerely thank those who donated their bodies to science so that anatomical research could be performed. Results from such research can potentially improve patient care and increase mankind's overall knowledge. Therefore, these donors and their families deserve our highest gratitude.

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